

**POPULATION ECOLOGY AND HARVESTING
SUSTAINABILITY OF *NEOPICRORHIZA
SCROPHULARIIFLORA* IN NEPAL HIMALAYA**



**A THESIS SUBMITTED TO THE
CENTRAL DEPARTMENT OF BOTANY
INSTITUTE OF SCIENCE AND TECHNOLOGY
TRIBHUVAN UNIVERSITY
NEPAL**

**FOR THE AWARD OF
DOCTOR OF PHILOSOPHY
IN BOTANY**

**BY
MUKTI RAM POUDEYAL
JUNE 2024**

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TRIBHUVAN UNIVERSITY
Institute of Science and Technology
DEAN'S OFFICE

Kirtipur, Kathmandu, Nepal

Reference No.:



EXTERNAL EXAMINERS

The Title of Ph.D. Thesis: " Population Ecology and Harvesting Sustainability of *Neopicrorhiza scrophulariiflora* in Nepal Himalaya "

Name of Candidate: Mukti Ram Poudeyal

External Examiners:

- (1) Prof. Dr. Mukesh Kumar Chettri
Amrit Campus
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- (2) Prof. Dr. Pooja Singh
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- (3) Prof. Dr. Niels Strange
University of Copenhagen
Copenhagen, DENMARK

June 25, 2024

(Dr. Surendra Kumar Gautam)
Asst. Dean

DECLARATION

Thesis entitled “**Population Ecology and Harvesting Sustainability of *Neopicrorhiza scrophulariiflora* in Nepal Himalaya**” which is being submitted to the Central Department of Botany, Institute of Science and Technology (IoST), Tribhuvan University, Nepal for the award of the degree of Doctor of Philosophy (Ph.D.), is a research work carried out by me under the supervision of Prof. Dr. Suresh Kumar Ghimire, Central Department of Botany, Tribhuvan University, Nepal, and co-supervised by Prof. Dr. Henrik Meilby, University of Copenhagen, Denmark, and Prof. Dr. Bharat Babu Shrestha, Central Department of Botany, Tribhuvan University.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

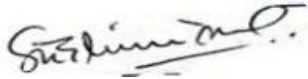


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Mukti Ram Poudeyal

RECOMMENDATION

This is to recommend that **Mr. Mukti Ram Poudeyal** has carried out research entitled **“Population Ecology and Harvesting Sustainability of *Neopicrorhiza scrophulariiflora* in Nepal Himalaya”** for the award of Doctor of Philosophy (Ph.D.) in **Botany** under our supervision. To our knowledge, this work has not been submitted for any other degree.

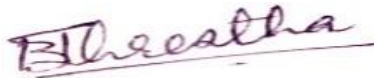
He has fulfilled all the requirements laid down by the Institute of Science and Technology (IoST), Tribhuvan University, Kirtipur for the submission of the thesis for the award of Ph.D. degree.



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June 2024



TRIBHUVAN UNIVERSITY
INSTITUTE OF SCIENCE AND TECHNOLOGY
CENTRAL DEPARTMENT OF BOTANY

Ref No:

Kirtipur, Kathmandu
NEPAL

LETTER OF APPROVAL

Date: 30/06/2024

On the recommendation of **Prof. Dr. Suresh Kumar Ghimire**, **Prof. Dr. Bharat Babu Shrestha** (Central Department of Botany, Tribhuvan University, Kirtipur, Kathmandu), and **Prof. Dr. Henrik Meilby** (Department of Food and Resource Economics, University of Copenhagen, Denmark), this Ph.D. thesis submitted by **Mukti Ram Poudeyal**, entitled “**Population Ecology and Harvesting Sustainability of *Neopicrorhiza scrophulariiflora* in Nepal Himalaya**” is forwarded by Central Department Research Committee (CDRC) to the Dean, Institute of Science and Technology (IoST), Tribhuvan University.

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Sangeeta Rajbhandary, PhD
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Central Department of Botany
Tribhuvan University
Kirtipur, Kathmandu, Nepal

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I am profoundly grateful to the local peoples (particularly our beloved porters and arrangers: Mr. Amar Bista and Mr. Bamboo Tamang) of the Darchula and Rasuwa, Nepal, who shared knowledge and provided transportation and logistic support in such magical remote mountain terrains. I am also thankful to the administrative staff of the CDB TU, Danida Fellowship Center, Copenhagen, Denmark, and Department of Food and Resource Economics, University of Copenhagen, Demark, for working space, logistic support, and assistance in different course-works.

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Last, but not least, I would like to remember all my friends who directly and indirectly have supported, helped, and encouraged me.



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शोध-सार

वनजन्य वनस्पतिहरु मध्ये विशेषगरी औषधिजन्य जडिवुटिको पास्थितिक, सांस्कृतिक तथा आर्थिक महत्व रहेको हुन्छ । संसारभरिनै जडिवुटिको परम्परागत स्थानिय औषधोपचार तथा बेचबिखनबाट हुने नगद आम्दानीलाई ग्रामिण आधारभूत आवश्यकता र जनजीविकाको आयआर्जनको मेरुदण्डको रूपमा लिइन्छ । यसको दिगो संकलन र व्यवस्थापनलाई गरिबी निवारण तथा वनको संरक्षण सम्बर्धन गर्ने महत्वपूर्ण औजारको रूपमा पनि लिईएको पाईन्छ । तर जब औषधिजन्य जडिवुटि वनस्पतिको व्यापारिकरण संगसँगै बढ्दो माग तथा बजार मूल्य र त्यस अनुसारको दोहन एउटै परिदृश्यमा देखा पर्दछ त्यसैसँगै आउने सामाजिक, आर्थिक तथा सांस्कृतिक अवयवहरुलाई समन्वात्मक तवरले समाधान खोजीनु अपरिहार्य रहन्छ । यिनै समस्या समाधानको शुत्राधारको लागि जडिवुटिको सामाजिक-पारिस्थितिक अवधारणालाई लिएर स्रोतको अनवरत उत्पादन, प्रयाप्तता तथा समुचित प्रयोगार्थको लागि सदियोंदेखि उपचार पद्धतीमा आईरहेको तथा राष्ट्रिय र अन्तरराष्ट्रिय व्यापारिकरण भई दोहन भईरहेको उच्च हिमाली वनस्पति कुट्की (न्योपिक्रोराईजा स्क्रोफुलारीफ्लोरा) को दिगो संकलनको विधि, धाचा तथा दिर्घकालीन व्यावस्थापन रणनीती तयार पार्न सहयोग गर्नु नै यो शोधकार्यको प्रमुख उद्देश्य हो । विशिष्टकृत रूपमा नेपालभरी पारिस्थिक प्रणाली अनुसार प्राकृतिक विचरणमा पार्ने प्रभाव, स्रोतको उपलब्धता, प्रयाप्तता तथा सुनिश्चितताका र त्यस अनुसारको संकलन, बेचबिखन र पैठारी रहेको वा नरहेको छुट्याउनु नै अध्ययनको पहिलो उद्देश्य थियो । त्यस्तैगरी भिन्न पारिस्थिक प्रणाली [उच्च (समुन्द्रि सतह बाट ४२५० मि. भन्दा कम) तथा न्युन (समुन्द्रि सतह बाट ४२५० मि. भन्दा बढि) हिमाच्छेदीत भूभागहरुमा] अनुसार विभिन्न स्तरको (०%, पूर्ण नियन्त्रण देखि १००%) कटान परिक्षण प्रयोग गर्दा विरुवाको जनसांखिक संरचना, प्रजनन क्षमता, वृद्धिविकाश तथा समग्र जनसांखिक बनोटको उलट-पुलटमा पार्ने प्रभावको मुल्यांकन गर्ने र न्युन क्षतिमा उच्चतम लाभ लिने विधि पत्ता लगाउनु शोधकार्यको अर्को उद्देश्य थियो । यसका लागि नेपालको उत्तरी हिमाली भूभागमा स्थानिय प्रयोगको लागि तथा व्यापारिक कटान तथा संकलनका लागि खुल्ला अपिनाम्पा संरक्षण क्षेत्र र व्यापारिक संकलनका लागि प्रतिबन्धित लाइटाइड राष्ट्रिय निकुञ्जमा दिर्घकालिन प्लट बनाई सन् २०१५ देखि २०१८ सम्म परिक्षण अध्ययन गरियो । जि. आई. एस्. विधिको अध्ययन अनुसार नेपालमा कुट्कीको संभावित विचरण क्षेत्र ११६१७

स्क्वाएर कि. मि. (करिब ८%) रहेको छ । जसमध्ये पुर्वी तथा मध्य उच्च पहाडी भूभागको ३८६ स्क्वाएर कि. मि., करिब ०.३% मात्र उत्तम गुणस्तरीय वासस्थान पाईएको र समुन्द्रि सतहबाट ४००० मि. देखि ४४०० मि. को उचाईमा मात्र साघुरिएर रहेको पाईन्छ । वातावरणीय अनुकुलनताको आधारमा विरुवाले उसको उच्चतम वृद्धि हुने समय जस्तै जून देखि अगस्त महिनामा मध्यमस्तरीय तापक्रम र उचित मात्राको वर्षात हुने ठाँउमा राम्रो भएको पाईयो । छोटो तथा न्युन वर्षात र उच्च तापक्रमका कारणले गर्दा पश्चिम नेपालतिर वासस्थानको गुणस्तर घट्दै गएको पाईन्छ । बिगत १२ वर्षको सरकारी आँकडा अनुसार नेपालबाट जम्मा ३७२ टन (मूल्य: करिब ५९१५७० अमेरिकन डलर, औसतमा प्रतिवर्ष ३१ टन) निकासी हुने गरेको र सबै तौलको करिब ९२% पश्चिम नेपालबाट मात्र भएको पाईयो । अधिक न्युन गुणस्तरको वासस्थानको उपलब्धता तथा अधिक दोहनलाई सँगै जोडेर हेर्दा समग्र नेपालमा अहिलेको संकलन प्रणाली दिगो नभएको पाईएको छ । तथापि व्यापारिक रुपमा खुल्ला तथा प्रतिबन्धित क्षेत्रमा भएको भौगोलिक प्रत्यक्ष अध्ययनमा स्थानिय उपचारको लागि गरिने परम्परागत संकलन परिमाण दर उस्तै रहेको पाईएको छ । कुट्कीको जनघनत्वमा भैरहेको स्थानिय तदर्थ दोहनको प्रभाव मुल्यांकन गर्दा प्रजनन र अप्रजनन दुवै अवस्थाका विरुवामा खुल्ला क्षेत्रमा नकारात्मक असर रहेको तर प्रतिबन्धित क्षेत्रमा अप्रजनन अवस्थाका विरुवामा सकारात्मक असर रहेको थियो । मध्यम स्तरको दोहनले प्रजनन र अप्रजनन वृद्धिको छेकबार/बन्देजलाई बढाउने सेतुको रुपमा रहेको र जव दोहनको स्तर बढ्छ विरुवामा कोपिला लाग्ने दर रेट घटेको पाइयो । अतः विरुवाको जनसांखिक वृद्धि खुल्ला क्षेत्रमा अप्रजनन प्रसारणसँग र प्रतिबन्धित क्षेत्रमा प्रजनन प्रसारणसँग केहि सकारात्मक सम्बन्ध रहेको थियो । विरुवामा रहेको अप्रजनन प्रसारणजन्य गुणहरु जस्तै मौका मिल्दा हुने अप्रत्यासीत अंकुरण क्षमता तथा सामान्य अवरोधको बदलाभमा नयाँ नयाँ पालुवा अंकुरण हुने क्षमताको बिस्तार गर्न सक्ने शंचित शक्ति प्राकृतिक रुपमै भएकाले कुट्कीमा केहि हदसम्म कटानको नकारात्मक प्रभावलाई सहन सक्ने निष्कर्ष निकालियो । तथ्यांकीय विप्लेशण रिपिटेड मिक्स्ड इफेक्ट मोडलीङका अनुसार कटान संकलन पश्चात हुने जनघनत्व अंकुरण भरण तथा प्रजनन उत्पादन संकलन—कटान दरमा तथा कटान गर्नु भन्दा अगावैको जनघनत्वमा भरपर्ने र सकारात्मक सम्बन्ध रहेको पाईयो । प्रतिबन्धित क्षेत्रको न्युन (समुन्द्रि सतह बाट ४२५० मि. कम) हिमाच्छेदीत भूभागमा ५०% सम्म कटान गर्दा परिक्षण अवधिको तिन वर्षमै पुनःभरण भएको र २५% सम्मको कटान प्रत्येक वर्ष गर्दा पनि पुनःभरण हुने परिणाम

पाईयो । तर खुल्ला क्षेत्रको समान स्तरको भूभागमा उक्त अवधिमा २५% कटान मात्र पुनःभरण भएको पाईयो । कटान पश्चातको पुनःभरण क्षमता भौगोलिक उचाई बढ्दा तथा कटान प्रतिशत दर बढाउदा घटेको पाईयो । पुनःभरण क्षमता प्रतिबन्धित क्षेत्रको तुलनामा खुल्ला क्षेत्रमा धेरै ढिलो भएको पाईएको छ । अर्को तथ्यांकीय विष्लेषण म्याट्रिक्स मोडलीङ्का अनुसार लगभग सबै ठाँउमा विरुवाको जनसांखिक वृद्धिदर २५% सम्म कटानको दुई बर्षको अवधिमा सकारात्मक स्तर भन्दा माथि रहेको पाईयो । बिषेशगरी प्रतिबन्धित क्षेत्रको न्युन हिमाच्छेदीत भूभागमा ५०% सम्मको कटानले विरुवाको जनसांखिक वृद्धिदर उक्त अवधिमा कटान परिक्षण अगाडिको भन्दा अझ बढेको भेटिएको छ । तर खुल्ला क्षेत्रको उच्च हिमाच्छेदीत भूभागमा जनसांखिक वृद्धिदर कटान नियन्त्रित परिक्षण अवस्थामै पनि घटिरहेको हाम्रो अध्ययनमा पाईयो । विरुवाको जनसांखिक मुल्यांकन अनुसार परिपक्व उमेरको विरुवा छनौट विधिबाट गरिने कटान संकलन त्यति व्यावाहिक नभएको र प्रत्येक कटान समयमा कम्तिमा २०-२५% फूलफल लागेका विरुवा रहने गरी भएमा वा प्रत्येक जनसांखिक संरचनामा उक्त २०-२५% फूलफल लागेका विरुवा सुनिश्चित भएमा मात्र कटान अनुमति दिन मिल्ने देखिन्छ । कुट्कीको दिगो संकलन, क्षति न्यूनीकरण, र जनसांखिक सुनिश्चितताका लागि समय र ठाँउ अनुसारको रणनीतिक कटान-संकलन व्यावस्थापन गर्नुपर्ने र प्रत्येक चार बर्ष अन्तरालको अवधिमा मात्र कटान अनुमति दिन सकिने यो सोधकार्यको सुझाव रहेको छ ।

मुख्य शब्दावली: औषधीजन्य विरुवा; बासस्थान उपयुक्तता; स्रोतको प्रयाप्तता; संकलन तथा कटान सुपेरिवेक्षण; मिश्रित प्रभाव मोडलिङ; संक्रमण म्याट्रिक्स; स्थिरता

ABSTRACT

Wild plant resources, including especially medicinal and aromatic plants (MAP) have great ecological, cultural, and economic value and contribute significantly to people's livelihoods in many of the world's rural areas. Additionally, it is widely acknowledged that the sustainable use of MAPs derived from natural ecosystems is an essential instrument for enhancing rural economies and forest protection through poverty reduction. A number of connected issues of social, ecological, cultural, and environmental character must be addressed when the commercialization and sustainable use of wild MAPs are envisioned in a single paradigm. This thesis takes a step in that direction and uses a social-ecological approach to develop a model for sustainable harvesting of *Neopicrorhiza scrophulariiflora* which seeks to create consistency between production and the renewable resource supply from the Nepalese Himalayas. We concentrate on how ecological changes drastically altered plant distribution and how resource availability connects to usage patterns across Nepal (via trade and transportation systems). We tested whether the variation of harvest (experimental harvest: removal of 0-100% of the plants) and the ecological conditions (lower alpine, <4250 m, vs upper alpine, >4250 m) affect population structure, reproduction, growth rates, and population dynamics in commercially open and protected regions in northwestern and north-central Nepal. The area of the potential distribution of the species was calculated to be 11617 km², corresponding to 8% of Nepal's land area and primarily located in the eastern highlands. Particularly suitable habitat areas (386 km²; 0.3% of the land area) tended to lie in the narrow altitude range of 4000-4400 m above sea level, especially in the central and eastern regions. The plant species prefer mild temperatures and sufficient rainfall in the middle of the growing period (June-August), and such conditions often occur in the mentioned areas. On the other hand, decreasing rainfall and greater temperatures diminished the suitability of the habitat in the western regions where the populations are intensively harvested with a view to commercial exploitation. Over 12 fiscal years, available official records showed that a total quantity of dried rhizomes of 372 tons (31 tons/year) valued at USD 591570 was exported to other countries. Of this, 92% of the amount came from western Nepal, which indicates that the current resource utilization is not sustainable. In the protected area, the recent harvest, which was largely carried out by locals (de facto harvest), had a positive effect on population density for vegetative and

a negative effect on reproductive individuals, while it had a negative effect on all plant stages in the area where exploitation is unregulated. Harvesting involves a trade-off between vegetative regrowth (clones) and sexual reproduction, and flowering was reduced when harvesting intensity increased. Apparently, the growth rate of the populations is positive and occurs predominantly by clonal reproduction in the unregulated area and sexual reproduction in the protected area. Clonal behavior, such as fugitive recruitment and accelerated bud production, can help a plant mitigate the detrimental impacts of harvesting and, theoretically, boost the harvest potential. Mixed-effects statistical models were used to model data from simulated harvest experiments and showed that recovery of population density and sexual reproduction varied significantly depending on harvest treatments and pre-harvest plant density. In lower alpine populations at the protected site, both density and reproduction were restored within three years after harvesting up to 50% of the plants, and within one year after harvesting up to 25% of the plants. In contrast, recovery to pre-harvest conditions at the unregulated site was only achieved after one year of harvesting 25% of the plants. Post-harvest recovery was slower at higher altitudes (upper alpine, >4250 m) and plots with more intensive harvesting (>50% harvesting) recovered more slowly. Based on matrix modeling, it was found that the population growth rate (λ) when removing up to 25% of the plants made it possible to maintain the pre-harvest condition at a 2-year harvest interval in almost all the studied populations. In the protected area, population growth was unaffected by the removal of up to 50%, and instead, the growth rate increased more than in the control plots. At the high alpine sites in the unregulated area, however, the growth rate was decreasing, even on the control plots, and harvesting had a further detrimental effect on the populations, making it necessary to use relatively long time intervals between harvests to achieve stability. Selective harvesting of particular developmental stages was difficult to achieve, nevertheless, it is considered worthwhile to leave 20-25% of the fruiting ramets at each harvest if 25% of the plants have flowered. In general, we strongly recommend that in order to achieve sustainable harvesting spatio-temporal rotations be introduced, so that a time interval of at least four years is achieved between harvesting activities in a given area and so that the plants are given a minimum amount of time to mature.

Keywords: medicinal plants; habitat suitability; mixed-effects modelling; post-harvest monitoring; transition matrices; sustainability

LIST OF ACRONYMS AND ABBREVIATIONS

ACA	:Annapurna Conservation Area
AIC	:Akaike Information Criterion
ANCA	:Api-Nampa Conservation Area
ANCOVA	:Analysis of Co-variance
ANOVA	:Analysis of Variance
ANSAB	:Asia Network for Sustainable Agriculture and Bioresources
APG	:Angiosperm Phylogeny Group
AUC	:Area Under Curve-Receiver Operating Characteristics
AV	:Adult Vegetative
CAMP	:Conservation Assessment and Management Plan
CBS	:Central Bureau of Statistics
CDB TU	:Central Department of Botany, Tribhuvan University
CITES	:Convention on International Trade in Endangered Species
DDC	:District Development Committee
DFO	:Divisional Forest Office
DHR	:Dhorpatan Hunting Reserves
DNPWC	:Department of National Parks and Wildlife Conservation
DoF	:Department of Forests
DPR	:Department of Plant Resources
E	:Royal Botanical Garden Edinburgh Herbarium
F	:Fecundity
GBIF	:Global Biodiversity Information Facility
GCA	:Gaurishankar Conservation Area
glm	:Generalized Linear Modelling
GLMM	:Generalized Linear Mixed Effects Model
GoN	:Government of Nepal
HMG/N	:His Majesty's Government of Nepal
IUCN	:International Union for Conservation of Nature
JV	:Juvenile
KATH	:National Herbarium and Plant Laboratory
KCA	:Kanchenjunga Conservation Area
LA	:Lower Alpine
LMM	:Linear Mixed Effects Model
LNP	:Langtang National Park

m a.s.l	:Meter above the sea level
MAPs	:Medicinal and Aromatic Plants
MaxEnt	:Maximum Entropy
MBNP	:Makalu Barun National Park
MCA	:Manaslu Conservation Area
MoFSC	:Ministry of Forest and Soil Conservation
NRs	:Nepalese Rupees
NTFP	:Non-Timber Forest Products
P	:Progression
PADIR	:Potential Annual Direct Incident Radiation
Pas	:Protected Areas
R	:Retrogression
Rep	:Reproductive
RNP	:Rara National Park
ROC	:Receiver Operating Characteristics
RSFG	:Rufford Small Grants Foundation
S	:Stasis
SDM	:Species Distribution Model
SE	:Standard Error
SNP	:Sagarmatha National Park
SP	:Sprouting
SPNP	:Shey-Phoksundo National Park
SRTM	:Shuttle Radar Topographic Mission
SRTMDEM	:Shuttle Radar Topographic Mission Digital Elevation Model
TGGN	:Transiting to Green Growth Nepal
TI	:Tokyo University Herbarium
TRAFFIC	:The Wildlife Trade Monitoring Network
TSS	:True Skill Statistics
TUCH	:Tribhuvan University Central Herbarium
UA	:Upper Alpine
USD	:United States Dollar
UV	:Ultraviolet
VIF	:Variance Inflation Factor
WGS	:World Geodetic System

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CHAPTER 1

1. INTRODUCTION

1.1 Background

The interaction between humans and their environment is intrinsic and firmly integrated with knowledge about and utilization of natural resources from the earliest days of history – and presumably also in prehistorical times. Such interconnection is more comprehensive and deeply embedded elsewhere in the world, where a significant fraction of people is devoid of diversifying livelihood opportunities and, thus, largely depend on goods and services derived from nature (Shrestha, 2014; Smith-Hall *et al.*, 2018). Environmental products are the safe and effective immediate source of food, medicines, fiber, construction materials, and artifacts for those people. Out of all, non-timber forest products (NTFPs) and their derivatives are the most exploited environmental goods (Hamilton, 2004; Ticktin, 2015). However, before 1980, NTFP appeared as neglected or less valuable or sometimes declared as forest minor/side products in relation to the contemporary monetary value of timber products in the mirror of policymakers and even to academic practitioners in many parts of the world. Sometimes, the consequential less negative impact of NTFP collection on the forest ecosystem in contrast with timber logging might be the reason to bring it into a priority level discussion.

In the late part of the 1980s, the value and services provided by the NTFP were widely regarded as a bridge for viable socio-cultural runs, affordable health care system, and sustainable economic development, specifically for the marginalized section of society (Ticktin, 2015; Shackleton *et al.*, 2018). As many findings declared, NTFPs are the source of multiple livelihood securities: such as provisioning to consumption for supplemental and complementary forms and safety nets to combat hunger, regulating cash income through selling and substituting the economic shocks, and finally, supporting and maintaining cultural tradition and social cohesion (Shackleton *et al.*, 2018; Pyakurel *et al.*, 2018). Majority of cases, harvesters are the primary users of those products. A number of studies (Angelson *et al.*, 2014; Antonelli *et al.*, 20; Rahman *et al.*, 2021) show that both consumable and non-consumable values of NTFP per unit area were found to be higher than the timber products in the world. However, these studies have received limited attention; thus, the sustainable rate

of extraction and wise management can provide the opportunity to conserve vital components of biodiversity.

Any items derived from the forest environment that are not wood from trees are considered non-timber forest products (NTFP). Examples include edible plants, medicinal and aromatic plants, spices, fatty oils, gum and resin, tannin, dye, fiber, fodder, saponin, honey, plumage, bones, musk, and silk. Additionally, the most well-known and extensively studied NTFPs are the medicinal and aromatic plants (MAPs), which should be given global priority for conservation and management (Hamilton, 2004). In a technical sense, the term 'medicinal and aromatic plants' refers to a group of plants that are used for both therapeutic and fragrant reasons, as well as a variety of food products, natural ingredients for condiments and cosmetics, and curative agents (Schippmann *et al.*, 2002; Hamilton, 2004). As a source of primary healthcare, globally, as many as four billion (almost 50%) people from rural depend on herbal products valued at almost USD 100 billion (Antonelli *et al.*, 2020).

Societal and cultural preferences mediate MAP's contribution to these functions and knowledge domains, and influence MAP's use and distribution in terms of which species are selected and by whom, when, how, and where they are located (Shackleton *et al.* 2018). In the past, the collection and harvest of MAPs were restricted to the traditional healers/gatherers, and gradually value of conventional treatment was replaced by modern pharmaceuticals. However, as a result of urbanization, and at present, mainly due to limited side effects compared to synthetic drugs, urban society is again inclined to herbal products; hence, the market demand is increasing at present (Smith-Hall *et al.*, 2018; Pyakurel *et al.*, 2018). So, the MAPs harvest from the wild became a significant domain to the unskilled and untrained commercial gathers of the rural worlds who have limited cash income for livelihood support. The social-ecological systems that make up MAPs are appropriately complicated and fit within a common framework (Larsen and Olsen, 2007). In the meantime, all the societies in every part of the world, and all the species harvested for economic values, are not managed or harvested in a sage way; and are heavily influenced by socio-economic dynamics (Ghimire *et al.* 2004; Shackleton *et al.*, 2018). The biological response and resilience of such exploited resources may vary between species. Nevertheless, the right fact is that the present use of consumption plants as medicine is indispensable for human livelihood. The dark reality is that the majority of harvesting practices are thought to be unsustainable and destructive for the long-term damaging in the meantime.

Broadly, significant challenges for the sustainable harvests of MAPs population can be categorized into i) limited ecological knowledge on use and management practices, ii) inadequate economic returns, iii) lack of evidence-based policies and their implementation, and iv) preliminary research. The sustainability of MAPs can not be dealt with in isolation; instead, inclusive approach of social and environmental paradigms is needed. For example, overexploitation to meet the commercial demand by immature and destructive collection even for local use is a rampant threat (Ticktin, 2015; Shackleton *et al.*, 2018). Commercial harvest causes exploitation, and sometime legal framework may not fit the social needs and species' ecological requirements. In the Nepalese Himalayas, geographical constraints such as steep terrain and mountain barrier and limited space for agricultural production create a high dependency on natural resources like in MAPs. Poor diversifying economic opportunities, little infrastructural development in highlands area, and substandard socio-cultural life exacerbate the exploitation of trade-related MAPs (Uprety *et al.*, 2016).

Every year, a significant number of research findings related to Himalayan plant resources for medicinal use are published, and most of them are sporadic, unfocussed, and lacking in-depth information for policy development (Kunwar *et al.*, 2013; 2020). Besides, how the MAPs (particularly at the species level) are intricately related to the Nepalese economy, what environmental factors sustain growth dynamics and supply, which areas support sustainable harvest, and which management strategies suit along the continuum of the environment are largely lacking (but see Larsen and Olsen, 2007). Assessment of the possible threat, prioritization of habitat regions for protection, and restoration of threatened ecosystems all depend on comprehensive knowledge about the species' biological niche and distribution (Dhar *et al.*, 2000; Chitale *et al.* 2014; Rana *et al.*, 2017). To bridge that gap, we assessed: i) environmental consequences of population growth and maintenance, ii) local level of harvest rate, suitable areas for harvest, economic returns, and trade channels, iii) ecological impacts of experimental harvests such as reproduction, survival, and mortality rate for the case MAP species *Neopicrorhiza scrophulariiflora* (Pennell.) Hong. (*Neopicrorhiza* hereafter) in some localities of the central and western mountains of the Nepalese Himalayas. **Appendix A** provides historical information, taxonomic details, and species identification, including those of additional closely related species. The whole plant is harvested for its indigenous medicinal use. It has a century-long transboundary trade history throughout the Himalayas (Forthcoming chapters provide the study species details).

The findings include novel insights to address the management problems of the *Neopicrorhiza scrophulariiflora*, and that can be applied to other Himalayan MAPs, particularly in support of resource extraction, supply, and ecological integrity.

1.2 Theoretical Basis and Rationale

Resource extraction for environmental products can be sustainable if it avoids long-term decline, preserving present demands and expectations for future demand (Ticktin, 2015). Fantini *et al.* (1992) established the idea of sustainable management of forest products (plant resources) and applied it to South American palms. If the current system of use meets the needs of the ecological, economic, social, and cultural communities, human use of biological resources, such as in MAPs, can be sustained. In order to preserve the ecosystem that is controlled by humans, these demands (represented by four domains in **Figure 1**) directly interact with one another in nature.

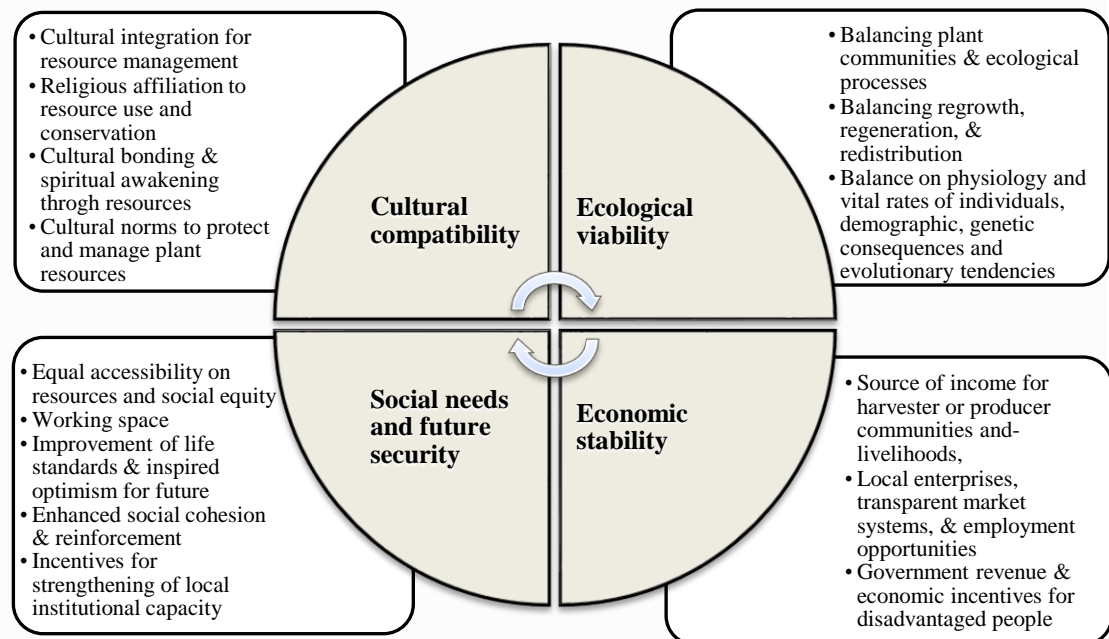


Figure 1: Revolving domains of sustainable harvesting of biological resources (different box sizes have no any indication)

A key necessity for achieving sustainable extraction is cyclical harvesting procedures and ecological dynamics of each harvested species. Such plant resources must be wisely utilized while maintaining and not jeopardizing the ecological integrity of nature (at each level: individual, population, and community). Within the plant community where exploitation is taking place, the harvesting process should not impede the ecological process or the natural evolutionary process therein (**Figure 1**). Socioeconomic stability is based on preserving the benefits of human welfare from those natural resources, including in the cultural and religious realms, while distributing

financial incentives equally (**Figure 1**). The dynamic character of socio-cultural, ritualistic, and spiritual values passed down through generations within the folk should not be disrupted while using resources (Ghimire *et al.* 2004). Overall, the resource utilized should help the government increase its tax collection and, ultimately, should improve the nation's economy and level of living for its citizens.

Globally, an increasing number of the human population has relied on MAPs and related products for various needs since the early days of civilization. In the meantime, under wise and tactful management conditions, resource exploitation in wild habitats still seems adequate for fulfilling present demands (Ticktin, 2015). However, commercialization, particularly for traded MAPs lead to unsustainable harvesting which reduces resource and poses a serious threat to stability, specifically to the sensitive and fragile ecosystems, like in high-altitude Himalayan environment (Ghimire, 2008). A thorough analysis should be conducted for raising their standards of living and social status in developing countries like Nepal, where MAPs are essential to maintaining rural residents' means of subsistence. Special attention should be paid to distribution patterns, the availability of resources, current exploitation approaches, and their sustainability for future generations.

The harvesting of MAPs at any level alters the ecological integrity of the plant population (He *et al.*, 2011; Ticktin, 2015). However, sustainable harvesting may be varied on the plant parts that are harvested and the life history of the harvested individual, including survival, growth, and reproduction (Ticktin, 2004; Gaoue *et al.*, 2014). Changes in such vital rates alter the plant populations' demographic and genetic patterns and affect populations' dynamics (Gaoue *et al.*, 2014). The exact magnitude of this impact depends on the distribution in various types of habitats, regenerating capacity, the density of the population, and the nature and intensity of harvesting (Ticktin *et al.*, 2002; Mandle and Ticktin, 2012; Schmidt and Ticktin, 2012). In some cases, the plant density of herbaceous perennial species is positively correlated to periodical mild-harvest, but increasing harvesting levels reduce the recruitment and survival rates (Ghimire *et al.*, 2005 and 2008a; Huai *et al.*, 2013). Similarly, in palm plants, the partial removal of non-reproductive plant parts at certain times within the rotation results in increased or unchanged levels of growth and reproduction (Freckleton *et al.*, 2003; Endress, 2004; Gaoue *et al.*, 2011). The recovery of exploited tree species populations can be enhanced by operating intermediate levels of silvicultural practices and maintaining optimal growing conditions (Verwer *et al.*, 2008; Gaoue *et al.*, 2014).

In these plant species, the harvest may alter individuals' physiology and vital rates, however, after removing plant parts, they redirect resources to growth and reproduction by using stored reserves (Gaoue *et al.*, 2011; 2014).

The traditional management techniques and local regulations did not affect population dynamics when plant populations were harvested (Schmidt and Ticktin, 2012). However, in the case of Himalayan MAPs, market-prone harvesting practices in recent years dramatically influences the traditional harvesting regimes and management systems (Ghimire *et al.*, 2006; Huai *et al.*, 2013). Therefore, proper estimation of harvest levels based on the nature of the plant species, extraction of plant parts, biological impacts of harvest, their sustainability of extraction, and demographic resilience to harvested populations are fundamentally important.

The value of medicinal plants and the importance of conserving them is widely recognized throughout the Himalayas. There are not many studies, and those that exist are limited to a few species, on the economic and ecological consequences of unsustainable high-altitude MAP harvesting (Ghimire *et al.*, 2005; 2008; Manish and Pandit, 2019; Rana *et al.*, 2020). In this dissertation, species distribution modeling across the nation was combined with an empirical investigation on population dynamics and experimental harvesting in Langtang National Park (Central Nepal) and Api-Nampa Conservation Area (Far-western Nepal) for the indigenous Himalayan medicinal herb *Neopicrorhiza scrophulariiflora*. We attempted to describe the conditions and ecology characterizing populations' distribution and examine and explain the role of environmental factors and exploitation in the dynamics of these populations. The species is grazing tolerant, unpalatable to the livestock avoided if alternatives herbaceous species are available, and harvest is the means of anthropogenic threat to the vegetation at those localities (Ghimire *et al.*, 2006).

1.3 Research Philosophy

The study is primarily demonstrated by quantitative sampling that describes the distribution and consumption pattern, the current harvest and trade rate, and the plant response to harvest through *in situ* simulated harvest trials. The results are consistent with positivist scientific philosophy since they are based on observable and quantifiable facts that interpret the real world (Creswell, 2003; Sarkar, 2014). MAPs are currently being harvested and used by the local populations for both local therapeutic purposes

and for market sale. In order to produce quantifiable data, direct field observation, and monitoring techniques were used. Utilizing descriptive and inferential statistical methods, the deductive method is used, the information that is already known is verified, and the outcomes are then interpreted.

1.4 Hypothesis, Objectives, and Research Framework

The environment has a significant spatial impact on how populations of specific plant species are distributed. Plant postharvest recovery rates, environmental conditions, and harvest demand for local and commercial usage all vary in space and time. We, therefore, put up the hypothesis that the sustainability of the harvest of medicinal plants is governed by factors such as climatic suitability, harvest frequency and intensity, the recovery period following harvest, and spatial management of plant populations. Each chapter of the dissertation was given its own specific hypothesis under this general one, and the validity of each hypothesis was examined by means of appropriate research questions (**Table 1**).

Our overall objective was to establish a sustainable harvesting model that balances the ecological and socioeconomic values of *Neopicrorhiza scrophulariiflora* in the Nepalese Himalayas. The socioeconomic relationship and environmental elements function as extrinsic factors (plant-people) that control and promote plant populations. Plant colonization, evolutionary tendencies, and self-rejuvenation abilities are inherent elements (intrinsic factors) that interact with those extrinsic factors to regulate plant populations. While both intrinsic and extrinsic factors work together to regulate plant populations.

The research framework, which is comprised of four of each result-oriented hypothesis and is supported by associated research questions, is specifically provided in **Table 1**. A specific objective is developed to answer each of those questions, and a brief methodological roadmap is provided. The first and second specific research questions are individually answered in chapters two and three. Chapters four and five jointly contribute to answering the remaining two specific research questions

Table 1: Research framework covering specific objectives, research questions, hypothesis, and methods

Hypothesis	Research question	Specific objectives	Methods	Linked chapter/s
Overharvesting and heavy trade in climates not conducive to growth could result in the extinction of the local populations, which could provide sustainability problems for long-term management.	How do the targeted species differ in terms of spatial distribution along environmental gradients? Is the resource availability align with exploitation pattern?	to provide the proximal area of distribution and suitable habitats over the entire geographical boundary of Nepal, elucidating factors that affecting habitat suitability, and examine spatio-temporal pattern of exploitation to identify current problems on harvest	Species distribution modelling with occurrences GIS modelling and analysis of trade-collection history through export recorded by the government agencies	2
Plant response to harvest impact vary along the environmental gradients	How do populations in different environmental conditions and harvesting regimes differ in plant density and biomass production?	to identify the variables that affect population size, structure, and biomass of plants throughout a range of environmental conditions.	Evaluated biomass variability, density, and population structure along elevation gradients in two protection regimes, including the plant-use systems	3
The impact of exploitation on the sustainability of harvesting and the longevity of the plant population is largely dependent on the amount, timing, types, and methods of harvesting	What are the main factors determining the sustainability of harvest?	to measure the population recovery rate after an <i>in situ</i> harvest on plant density and their consequential effect on fitness-related traits through an annual census	Applied <i>in situ</i> simulated harvest field experiment in eight populations along an elevation gradient, plant density and reproductive outputs treated with the harvest treatments.	3 and 4
Not all the levels of harvest may be detrimental to the plant's fecundity, regeneration, and recruitment; and spatial and temporal response to plant extraction may determine the fate of the population and optimal harvest limit.	What are sustainable harvest levels for populations in different environmental conditions that can mitigate the problems on sustainability of harvesting?	to evaluate population dynamics across the gradient of experimental harvests and to identify the demographic properties that contribute to the population growth along the continuum of environmental conditions so as to provide optimal harvest limit.	Conducted analysis of population dynamics from annual 4-years postharvest records from eight plant populations and calculated fecundity and regeneration, survival, population growth and mortality rate	3 and 4

1.5 Structure of the Dissertation

The dissertation's organizational principles are based on the research regulations established by the Institute of Science and Technology at Tribhuvan University in Nepal (2021). Chapter one contains the background of the study, the problem statement, the hypothesis, and the summative goals of the dissertation. In chapter two, we asked whether the existing trade matches the geographical distribution of habitats and whether they have specific ecological determinants to quantify the occurrence across the entire

plant distribution range in Nepal. We identified major climatic variables that determine the habitat suitability, traditional trade pattern and export quantity, and inter-relations between existing exploitation patterns and potential areas of occurrence, which can be crucial for sustainable management and the development of farming and cultivation. Moreover, we discussed how the interplay between harvest, trade, and habitat suitability might be used to resolve sustainability and conservation challenges and to help local collectors by providing a means of fending off the growing demand from trade. The findings could be the best framework for policy development.

Chapter three deals with the impact of environmental factors including existing local harvest effects on plant density and biomass across the study sites and along with an elevation gradient. Specifically, we ask whether plant response to harvest varies with vegetation and substrate conditions. Our study showed that the low harvest intensity is tolerable for regeneration and allows maintenance for the plant populations. The response varies with plant life-history structure and habitat types. The baseline ecological problems and weaknesses dealt with the present harvest became a way forward to finding a sustainable harvest level.

In chapter four, we present long-term demographic information on harvest recovery. Our methodology for multi-year tracking of the effects of experimental harvesting on density and reproductive fitness traits across the environmental gradient offers urgent and timely insights for sustainable management. At the same time, our control, and empirical methods are reproducible in other systems, and our conclusions — that sustainable harvest is possible, but only under certain temporal and spatial conditions — are broadly applicable.

Similarly, chapter five presents population dynamics and growth rate through matrix modelling, where up to 50% removal of plants in a regulated condition at three years' harvest rotation did not affect regeneration rates, or population viability, and thus constituted a sustainable extraction approach. Chapter six is concluded with a synthesis of the preceding chapters and outlines the managerial and future implications of the research. We explained the contributions of our findings about conservation, discussed the limitations of our results, and recommended future lines of research.

CHAPTER 2

2. RESOURCE AVAILABILITY AND EXPLOITATION¹

2.1 Abstract

Despite the widespread use on traditional herbal medicine and trade of *Neopicrorhiza scrophulariiflora*, a high-valued medicinal plant species from the Himalaya, there is still inadequate information on its distribution and driving environmental factors for suitable habitats — needed to identify potential areas for sustainable resource extraction. We hypothesized that overexploitation and high trade in areas climatically less suitable for the growth might cause extinction of the local populations, which may lead to unsustainability issues for long-term management. To address this hypothesis, we conducted species distribution modeling of *N. scrophulariiflora* using ten environmental variables, and Maximum Entropy algorithm based on species occurrence records from Nepal, and finally, relate the statistics of 12 fiscal years' (2004–2016) trade assessment. The predicted suitable habitat areas in Nepal was estimated at 11617 km², and was limited to a narrow elevational range (4000–4400 m), in which only about 386 km² (0.3%) of the total area was highly suitable, predominantly located in eastern mountains, due to mild temperature and adequate precipitation at plants' peak growing months. Official records provided that Nepal exported 372 tons, unexpectedly low volume of *N. scrophulariiflora* rhizomes in 12 years' period (average: 31 tons/annum), mostly from western mountains where predicted habitats are less suitable. Discordant geographical patterns of habitat suitability, redundant extraction from low suitable habitats, and increasing trade indicate that existing resource exploitation is likely to be unsustainable. We suggest formulating management strategies for locations with heavy collection and trade, conducting cultivation trials in suitable patches, and identifying sustainable annual harvest limits.

Keywords: medicinal plants; maximum entropy; habitat suitability; traded volume; economic contribution; sustainable conservation

¹**Published Research Article:** Poudeyal, M.R., Pyakurel, D., Rana, S.K., Meilby, H., Paneru, Y.R., Ghimire, S.K. (2021). Does resource availability coincide with exploitation patterns? Inference from distribution and trade of *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong in the Nepalese Himalaya. *Journal of Applied Research on medicinal and Aromatic Plants*, 22:100292. <https://doi.org/10.1016/j.jarmap.2021.100292>.

2.2 Introduction

Medicinal plants in mountain ecosystems are an important basis for traditional health care systems and offer an opportunity to generate cash income in rural areas of developing countries (Hamilton, 2004; Jensen and Meilby, 2008). However, a significant number of medicinal plants in high mountains are confined within a narrow habitat range and are vulnerable to environmental change, habitat loss or modification, and destructive commercial harvest (Hamilton, 2004; Cunningham *et al.*, 2018a; Applequist *et al.*, 2020). As a result, to conserve such species, it is important to maintain the stability of ecologically important habitats, and an urgent research effort is needed to evaluate their risk of extinction. To do this, it is necessary to understand the species' ecological niches, examine their spatial distributions, assess potential threats, prioritize habitat areas for conservation, and to restore threatened ecosystems (Manish and Pandit, 2019; Rana *et al.*, 2020). Globally, species conservation and management decisions are largely based on known sites of occurrence, either collected through direct field observations or indirectly based on specimens deposited in museums and herbaria (Phillips and Dudik, 2008; Rana *et al.*, 2020; Nagahama and Bonino, 2020). Estimation of the potential area of distribution and suitable habitats of mountain plants using climatic and topographic variables is imperative to assess their vulnerability to changing environmental conditions, whether caused by natural or anthropogenic factors. Species Distribution Models (SDM) could be a useful way to obtain estimates of spatial distribution, potential abundance, and environmental adaptability, to find appropriate harvesting locations, and to develop effective plans for sustainable conservation (Shrestha and Bawa, 2014; Nagahama and Bonino, 2020). Moreover, SDM is considered as a cost-effective tool for providing information on ecological characteristics, factors shaping populations and connectivity, and habitat requirements within a relatively short time (Poudel *et al.*, 2012; Sharma *et al.*, 2018).

'Kutki' is a trading name given for four different species of commercial medicinal plants, globally recognized by two Himalayan genera: *Neopicrorhiza* [*N. scrophulariiflora* (Pennell) D.Y. Hong and *N. minima* R.R. Mill)] and *Picrorhiza* [*P. kurrooa* Royle ex Benth. and *P. tungnathii* Pusalkar] (Hong, 1998; Pusalkar, 2014). Rhizomes of all four species are used in traditional medicine. All four species have localized distributions in a narrow band of subalpine–alpine areas of India, Nepal, Bhutan, Tibet, and western China at 2700–5000 m above the sea level. Among the four

species, *P. kurrooa* and *N. scrophulariiflora* have been traded for millennia, thereby offering an opportunity to generate additional income for rural households in the Himalaya (Olsen, 2005; Uniyal, *et al.* 2011; Sharma and Kala, 2018). However, due to the inclusion of *P. kurrooa* in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II in 1997, the trade pressure has shifted towards *N. scrophulariiflora* (Mulliken, 2000). In Nepal, Kutki is represented by a single species, *N. scrophulariiflora* (Smit, 2000; Olsen, 2005; Ghimire *et al.*, 2005; Shrestha and Jha, 2009; Poudeyal *et al.*, 2019). It is considered as highly threatened species in Nepal due to excessive commercial harvesting, presumably beyond its regeneration capacity (Bhattarai *et al.*, 2002; Ghimire *et al.*, 2005). The traditional systems of harvesting, which incorporate low intensity of rhizome harvest for subsistence use at intervals, limit the impact at the local level (Ghimire *et al.*, 2005), but overharvesting to meet the high demand of lucrative markets seems problematic, even at the local level (Poudeyal *et al.*, 2019).

The annual supply of ‘Kutki’ rhizomes from Nepal, Bhutan, and India to the global market was estimated at 650–1000 tons, 50–300 tons of which was contributed by *P. kurrooa* and the rest was by *N. scrophulariiflora* (Olsen, 2005; Uniyal *et al.*, 2011). Nepal is the largest global supplier of Kutki rhizomes, the production amounts 175–770 tons per annum and covers 66% of the global market (Olsen, 2005). Recently, Pyakurel *et al.* (2018) reported that the export of high-value medicinal plants from the western Himalaya of Nepal has increased two times by volume and 17 times by value within the last two decades. The trade network is characterized by low transparency, only a little information is available on harvesting and trade, and no comprehensive biophysical studies are conducted before issuing harvest permit (Uprety *et al.*, 2016; Devkota *et al.*, 2017; Pyakurel *et al.*, 2018). Therefore, to increase the chances of maintaining supplies and achieving sustainable management in the future, reliable information on species occurrence and extraction potentials are urgently needed.

Despite its importance in traditional and modern medicines, and the increasing volume of trade and rising prices, the distribution and habitat requirements of *N. scrophulariiflora* have not been sufficiently studied. However, its distribution has been reported to be largely affected by species demographic constraints, harvest, and habitat exploitation and destruction, and changing climatic conditions (Ghimire *et al.*, 2005; Rana *et al.*, 2017; Poudeyal *et al.*, 2019; Sharma and Kala, 2018; Applequist *et al.*, 2020). In this research, we attempted to bridge the knowledge gap by identifying factors

affecting habitat suitability, estimating the distribution and area of suitable habitat, and examining the trade of Kutki from the Nepalese Himalaya to elucidate current problems and to inform sustainable management. We hypothesize that overexploitation and high trade in the areas climatically less suitable for the growth of *N. scrophulariiflora* might cause the extinction of the local populations, which may lead to unsustainability issues for long-term management. Identifying areas with optimal habitats and suggesting production alternatives (e.g., cultivation) may counteract the over-exploitation and supplement the resource. We sought to find the answers to the following questions: (1) What are the potential areas of suitable (high, medium, and low) and unsuitable areas, as habitats for *N. scrophulariiflora* within its natural range in Nepal? Does the habitat suitability vary across geographical regions? (2) What are the main ecological determinants that can be used to quantify the occurrence of the species within its entire range in the Nepalese Himalaya? (3) What is the current trade pattern in the country? (4) Does the trade align with the distribution pattern of plant species in the Nepalese Himalaya? To answer these questions, we evaluated the habitat suitability based on recorded occurrences of *N. scrophulariiflora* populations and used suitable bio-climatic variables to classify the habitats. Next, we compared the availability and geographical distribution of habitat suitability classes with information on trade patterns and revenue collection. We ensure that the result will provide novel insights into the trade-habitat interplay to address the sustainability issues in support of the medicinal plant conservation.

2.3 Material and Methods

2.3.1 Study Species

Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong (Synonym: *Picrorhiza scrophulariiflora* Pennell, Family: Plantaginaceae) is a rhizomatous perennial herb with fragmented distribution at 3500–4800 m a.s.l. in the Nepalese Himalaya. The plant bears multiple dark blue flowers in a long raceme, borne on a stout stem arising from a rosette of leaves. Fruit maturation and seed dispersal take place in September and October. Growth is slow, sexual reproduction is often rare, and expansion and colonization take place by vegetative shoots which help to develop clumpy patches (Ghimire *et al.*, 2005). Shrubland meadows dominated by *Rhododendron* species provide many favorable conditions for *N. scrophulariiflora* populations (Ghimire *et al.*, 2005; Poudeyal *et al.*, 2019). The species has not been assessed and assigned a global

conservation category, but at the national level, it was enlisted as ‘vulnerable’ by the Conservation Assessment and Management Plan (CAMP 2001) workshop (Bhattarai *et al.*, 2002) and has been banned for export without identification and certification by the Government of Nepal (HMG/MoFSC, 2001).

As a consequence of growing demand the competition among collectors is increasing and the commercial collection now mostly takes place before seed maturation, from August to October, thereby threatening the stability of wild populations (Ghimire *et al.*, 2005; Poudeyal *et al.*, 2019). Domestication of medicinal and aromatic plants (MAPs) is considered as an alternative to wild harvest to overcome the overexploitation. As a result trial cultivation of *N. scrophulariiflora* was initiated in different parts of the Himalaya but lack of detail understanding on climatic (temperature, precipitation), topographical (slope, aspect), and edaphic (soil nutrient) factors of the species makes the cultivation less successful (Ghimire *et al.*, 2005; Chandra *et al.*, 2006; Cui *et al.*, 2012).

Rhizomes of *N. scrophulariiflora* are used in Ayurvedic and Tibetan medicines as well as in folk healing system to treat a variety of ailments, including cold, fever, asthma, jaundice, malaria, high blood pressure, intestinal pains, conjunctivitis, urinary disorders, and snake bites (Lama *et al.*, 2001; Manandhar, 2002; Ghimire *et al.*, 2005; DPR, 2006). Acknowledging some of its useful properties, modern pharmacology has also adopted the species (Smit 2000; Liu *et al.*, 2011; Uniyal *et al.*, 2011; Kumar *et al.*, 2017). Rokaya *et al.* (2020) reported 124 compounds extracted from the rhizome, with iridoid accounting for the majority at 34.7%. Similarly, remaining compounds include phenol, cucurbitacin glycoside, hydroxycinnamate group, sugar and phenyl glycoside, fatty acid, alcohol, hydroquinone glycoside, coumarin, phytosterol glucoside, flavonoid and steroidal glycoside, and phenylpropanoid, all reported in decreasing order (Rokaya *et al.*, 2020). Stool hardness totally disappeared after the fifteenth day in a clinical trial made by Kohilathrshini *et al.* (2017) on the chemical components and health benefits of rhizome powder in patients aged 20 to 30 who had constipation issues. The patients took plant capsules (650g) twice a day for 40 days. Likewise, in patients treated with rhizome capsules prepared from boiling cow milk and dried in the shade for three days, there was a considerable improvement in prolonged straining, incomplete evacuation, lower abdominal fullness, and gas accumulation (Kohilathrshini *et al.*, 2017). According to the findings of these clinical studies, powdered plant rhizome is both safe and effective, with no negative consequences. Wider application of traditional medicinal

practices and recent pharmacological evidence regarding the species further increases its market value. Owing to its pharmacological uses and the associated trade potential, the Government of Nepal has prioritized *N. scrophulariiflora* for research and economic development, and for the development of agro-technological methods (DPR, 2006).

2.3.2 Study Area

A herbarium survey and government statistics showed that *Neopicrorhiza scrophulariiflora* is found and traded in sub-alpine and alpine areas within 20 mountain districts from eastern to western Nepal (**Figure 2**). Phyto-geographically, Nepal is divided into the western (80°04'–83°0' E), central (83°0'–86°30' E) and eastern (86°30'–88°12' E) regions (Stearn, 1960).

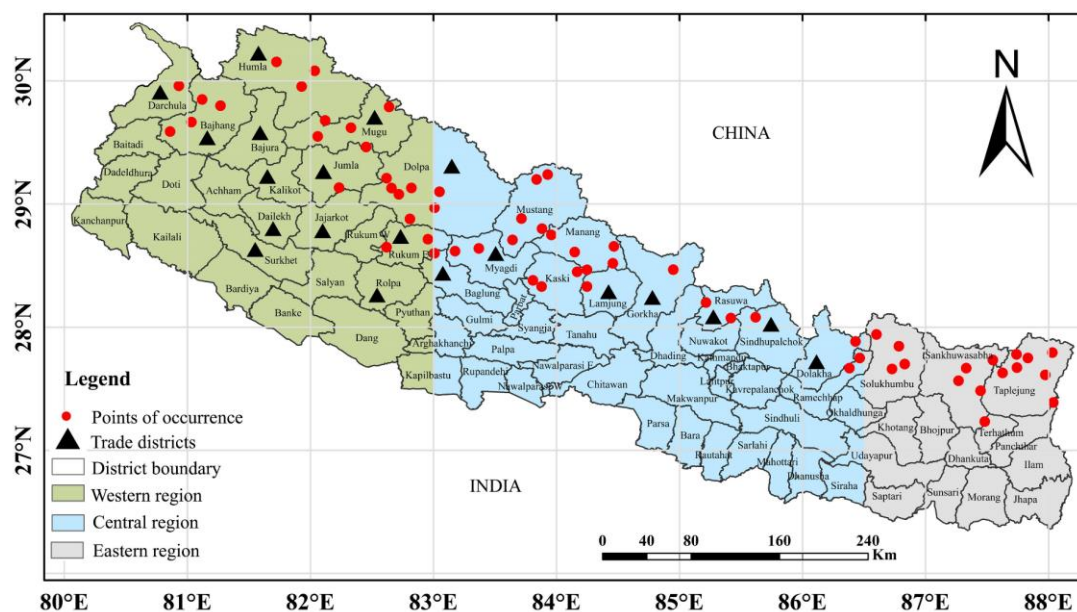


Figure 2: *Neopicrorhiza scrophulariiflora* distribution map indicating 20 districts in the Nepalese Himalaya as the source of herbarium specimens (herbarium survey) and trade history records. Three ecological regions are indicated by color zones (derived from (1960))

The monsoon lasts longest and the precipitation is highest in the eastern region, particularly at elevations >2000 m, and gradually decreases both in terms of duration and precipitation towards the western region (Miehe *et al.*, 2001; Böhner *et al.*, 2015). The large altitudinal variation within short distances (below 100 m in the southern lowlands to the 8848.86 m of Mount Everest within an average distance of 193 km), together with a large variation in annual rainfall and temperature, are the basis for a large diversity of habitats, and the country is home to a large number of medicinal plant species. Out of 1950–2331 species of MAPs reported from Nepal (Ghimire, 2008; Rokaya *et al.*, 2012), 300 species are harvested for trade (Pyakurel *et al.*, 2019). Twenty

species of commercial MAPs, including *N. scrophulariiflora*, cover almost 80% of the total traded volume (Heinen and Shrestha-Acharya, 2011), but the geographical distributions of the species have not yet been mapped or fully understood (but see Poudel *et al.*, 2012; Rana *et al.*, 2020; Kunwar *et al.*, 2020).

2.3.3 Species Location Data

Neopicrorhiza scrophulariiflora occurrence records were collected from direct field observations made by the authors; herbarium specimens deposited at the National Herbarium and Plant Laboratory (KATH) and Tribhuvan University Central Herbarium (TUCH), Nepal; online databases of Tokyo University Herbarium (TI; <http://umdb.um.u-tokyo.ac.jp/DShokubu/>), Royal Botanical Garden Edinburgh Herbarium (E; <http://data.rbge.org.uk/search/herbarium/>), Flora of Nepal (<http://www.floraofnepal.org/data>), Global Biodiversity Information Facility (GBIF; <https://www.gbif.org/>), and observations reported in published and unpublished literature. Most of the field observations were made by one of the co-authors (S.K. Ghimire) during field trips in 1995–2019. Additional geographical coordinates of *N. scrophulariiflora* populations were obtained from Lama *et al.* (2001), Manandhar (2002), Ghimire *et al.* (2005), Ghimire *et al.* (2008c), Ghimire *et al.* (2009), Shrestha and Jha (2009), and Poudeyal *et al.* (2019). Additional geographical coordinates of *N. scrophulariiflora* populations were obtained from Lama *et al.* (2001), Ghimire *et al.* (2008), Ghimire *et al.* (2009), Manandhar (2002), Ghimire *et al.* (2005), Shrestha and Jha (2009), and Poudeyal *et al.* (2019). Altogether, we collected ~250 occurrence points over the time between 1966 and 2019 covering locations across the entire range within Nepal. After the removal of duplicates, we ended up with a shortened list of 84 localities without duplication.

To reduce spatial correlation in the dataset, we used a spatial rarefaction tool in the SDMToolbox (version: 2.4) in ArcGIS, version 10.5 (Rana *et al.*, 2017; Rana *et al.*, 2020). A minimum filter distance of 10 km is considered appropriate in areas with high spatial heterogeneity, such as mountainous regions (Rana *et al.*, 2020). Therefore, the 84 short-listed locations were rarefied using a minimum distance of 10 km between neighboring locations to reduce the likely effects of spatial autocorrelation and the possible bias caused by over-sampled regions. This way we finally extracted 63 records, which we used to calibrate an SDM model to identify relationships between the occurrence of the species and a set of environmental variables.

2.3.4 Environmental Predictor Variables

Based on species occurrence data within the political boundary of Nepal and a base map of the country, a current suitable habitat map of *Neopicrorhiza scrophulariiflora* was produced. Along with a country map, we also calculated the area for three phytogeographic regions (**Figure 2**), the protected areas, and at the level of districts. Nineteen bioclimatic variables were derived from a global interpolated climate dataset (<https://worldclim.org/version2>; Fick and Hijmans, 2017) and used as potential predictor variables. In addition, since topographic variables are often believed to influence the distribution of plant populations (Shrestha and Jha 2009; Poudeyal *et al.*, 2019), we included three different topographic variables (elevation, aspect, and slope) as potential predictors of the distribution of *N. scrophulariiflora*. The bioclimatic variables were derived from average monthly climate data (maximum and minimum temperature, and total precipitation) from a reference period 1970–2000, prepared using a global set of weather stations and interpolated at a resolution of 30 arc seconds, corresponding to grid cells with an area of approximately 1 km² (Fick and Hijmans, 2017). The nineteen bioclimatic variables represent annual variation, seasonality, and extreme or limiting temperature and precipitation, all of which are potentially relevant to describe the eco-physiological conditions that determine the occurrence of *N. scrophulariiflora* populations. To derive topographic variables (aspect and slope) at the appropriate resolution we used a Digital Elevation Model based on the Shuttle Radar Topographic Mission (SRTM) (provided by United States Geological Survey), which we resampled using the nearest neighbor resampling technique. All environmental variables were thus available at a spatial resolution of 30 arc seconds (~1 km² grid resolution) (**Table B.1**).

The collinearity among the bioclimatic variables is high within relatively small geographic areas (Rana *et al.*, 2020). Using the ‘car’ package in the R-programming language (R Core Team, 2019; **Table B.2**) a variance inflation factor (VIF; Fox and Weisberg, 2011) was calculated with elevation as a response variable and used in combination with a Pearson correlation matrix to remove highly correlated variables. This was done to avoid multi-collinearity and over-parameterization of the model (Fox and Weisberg, 2011; Shrestha and Bawa, 2014; **Table B.3**). Hence, the final subset of predictor variables includes only variables with VIF values <10, and Pearson

coefficients of correlation <0.8. Consequently, we ended up with a set of predictors including only seven bioclimatic and three topographic variables (**Table 2**).

Table 2: Predictor variables used to calibrate the species distribution model for *Neopicrorhiza scrophulariiflora* in the Nepalese Himalaya

Geographical region	Nepal (Datum: WGS 1984)
Resolution	30 arc~ sec
Climatic variables	Attribute: Continuous; Source: Worldclim (Fick and Hijmans 2017)
bio3	Isothermality (bio2/bio7) (* 100)
bio4	Temperature Seasonality (standard deviation *100)
bio5	Maximum Temperature of Warmest Month
bio14	Precipitation of driest month
bio15	Precipitation seasonality (Coefficient of variation)
bio18	Precipitation of Warmest Quarter
bio19	Precipitation of Coldest Quarter
Topographic variables	Source: SRTM DEM (United States Geological Survey)
Elevation	Terrain elevation based on Digital Elevation Model (unit: meters)
Aspect	Aspect in degrees (cardinal direction, orientation of slope)
Slope	Slope in degrees (gradient for incline of surface)

Temperature is expressed in degrees Celsius, while precipitation is expressed in millimeters; (SRTMDEM: Shuttle Radar Topographic Mission Digital Elevation Model)

2.3.5 Species Distribution Modeling

Model calibration based on presence-only data is best done using the MaxEnt (Maximum Entropy, version 3.4.1) approach to predict the potential distribution of a species (Phillips and Dudik, 2008). Provided a dataset with sufficient coverage, it gives robust estimates of habitat suitability even with limited data and for species characterized by a patchy distribution with a narrow spatial extent, such as for *N. scrophulariiflora* populations (Phillips and Dudik, 2008; Rana *et al.*, 2020). To model the distribution of *N. scrophulariiflora*, MaxEnt used 25% random test and 75% training records from the original presence dataset with a single regularization multiplier against 10,000 randomly distributed background points. We calibrated the model using 50 replications with bootstrapping (Rana *et al.*, 2017), the default convergence threshold value of 0.00001, and a maximum of 5000 iterations. Moreover, we used linear, quadratic, product, threshold and hinge features of the MaxEnt algorithm (Shrestha and Bawa, 2014).

The predictive power of the MaxEnt model was evaluated using TSS (True Skill Statistics), Cohen's Kappa, and AUC (Area Under Curve-Receiver Operating characteristics) statistics (Allouche *et al.*, 2006; Rana *et al.*, 2019). TSS is independent

of prevalence, i.e. the ratio of presence to pseudo-absence data in the presence-absence predictions (Allouche et al., 2006). This statistic deals with both sensitivity and specificity and its value ranges from -1 to $+1$, where $+1$ indicates perfect agreement, and values ranging from 0.7 to 0.9 specify fair to good model performance (Allouche et al., 2006). The independent area under curve (AUC) statistic describes the model's discriminatory ability and was calculated from the receiver operating characteristics (ROC) (Rana et al., 2017). When examining model performance, AUC metric values between $0.5-0.7$ were considered as poor, $0.7-0.9$ moderate, and >0.9 as good (Manel et al. 2001). In addition, we used response curves to assess which variables had significantly affected the probability of the presence of *N. scrophulariiflora* (Phillips and Dudik, 2008). The Boyce index was used to evaluate how well the model distinguishes between suitable and unsuitable areas (Manly et al., 2002). Boyce index (Spearman's correlation coefficient) values of at least 0.90 were considered to indicate that the model accurately defined suitable areas. The potential habitat distribution was mapped using four suitability classes; unsuitable ($<25\%$ probability of presence), low ($25-50\%$), medium ($50-75\%$), and high suitability ($>75\%$ probability of presence) (Shrestha and Bawa, 2014, Rana et al., 2019; Kunwar et al., 2020). We used equal training sensitivity and specificity logistic thresholds to delimit suitable areas from unsuitable areas (Pearson et al., 2007). Using a base map of Nepal and potential habitat predictions from our model, we prepared a map of currently suitable habitats of *Neopicrorhiza scrophulariiflora*. This map was used in calculating the area of suitable habitats within three phytogeographic regions (**Figure 2**), within protected areas, and within individual districts.

2.3.6 Trade Information

Harvesters collect *Neopicrorhiza scrophulariiflora* from sub-alpine and alpine meadows and sell dried and cleaned products either to sub-local traders in villages, or to local traders at major road heads or district headquarters. Sub-local traders often receive a cash advance from local traders and never transport MAPs across district borders. Once the agreed volume (usually a tractor load) has been collected, local traders visit sub-local traders, settle advances and transport the products to their storehouse. Before this happens, the local trader receives a collection permit issued by the Divisional Forest Office (previously the District Forest Office) or the Conservation Area Office (in protected areas) and distributes copies to sub-local traders. Local traders

receive a transport permit based on the collection permit, transport the products out of the district to a central wholesalers' storehouse (see details in Pyakurel *et al.*, 2018; Smith-Hall *et al.*, 2018). Finally, wholesalers receive a phytosanitary certificate, pay the customs tax, receive a transboundary export permit, and export the products to India, China, or other countries. Department of National Park and Wildlife Conservation (DNPWC), Government of Nepal, and local authorities (e.g., Conservation Area Offices) regulate the medicinal plants trade from protected areas, whereas Department of Forests (DoF) and their local Divisional Forest Offices regulate the trade outside protected areas. These authorities record information on the quantity (volume) produced according to permits issued and the revenue collected.

For this study, we obtained DoF trade data from 12 fiscal years (2004/05 to 2015/16), which include information on the district of origin. We aggregated the district-level data to obtain estimates of national-level trade for each fiscal year. We correlated the suitable habitat area predicted by the SDM model with cumulative trade volume and annual trade frequency for the combined dataset including all districts to determine whether the existing exploitation pattern aligned with the predicted distribution of *N. scrophulariiflora*. The trade data from DoF (2006–2015) included records of a range of traded medicinal plants (50 to 70 species per year). For each year we calculated percentages of total volume (quantity) and value (revenue) to determine the contribution of *N. scrophulariiflora* to the overall trade in medicinal plants. We used price data from ANSAB (Asia Network for Sustainable Agriculture and Bioresources, <http://www.ansab.org/market-information/price-lists/>) to estimate market prices for *N. scrophulariiflora* in the five years from 2012 to 2016. We used price-lists for Kathmandu and Nepalgunj in Nepal for a national trend analysis, and for Delhi, Lucknow, Tanakpur and Kolkata in India for a regional trend analysis. The annual average price per kilogram was calculated as the average monthly price for each year and was multiplied by the average annual traded volume to estimate the annual trade value. We calculated prices in US Dollars (USD) using the annual average exchange rate provided by Nepal Rastra Bank (<https://www.nrb.org.np/>).

2.4 Results

2.4.1 Importance of Environmental Factors

MaxEnt modeling successfully delineated the potential distribution of *Neopicrorhiza scrophulariiflora* using seven climatic and three topographical variables (**Table 2**).

Notably, the topographic factors contributed more (68.0%) than the climatic factors (32.0%) to the model (**Figure 3a, 3c, and 3d**). Overall, elevation was the most influential predictor (51.4% contribution) of the distribution of *N. scrophulariiflora*, and the estimated probability of occurrence was high at elevations of around 4000–4400 m but decreased on either side of this range. The probability of occurrence was particularly high on slopes facing north-east and east, and with slope angles of 18–28° and 33–38°. The contributions to predicting the distribution were 8.7% and 8.2% for slope and aspect, respectively (**Figure 3c**).

Among the seven climatic variables, ‘maximum temperature of warmest month’ (bio5) turned out to contribute the most (second-most of all predictors) to the model (14%). Another important temperature-based variable was ‘isothermality’ (bio3), which contributed 6.2%. The probability of occurrence peaked at bio5 \approx 13°C (**Figure 3b**) but remained high within a range around this temperature. Among the precipitation-dependent variables, ‘precipitation of coldest quarter’ (bio19) contributed approximately 4% to the model and showed a peak value at 10 mm. The habitat suitability was also high at much higher precipitation levels and showed a second flat top at around 140–160 mm (**Figure 3f**).

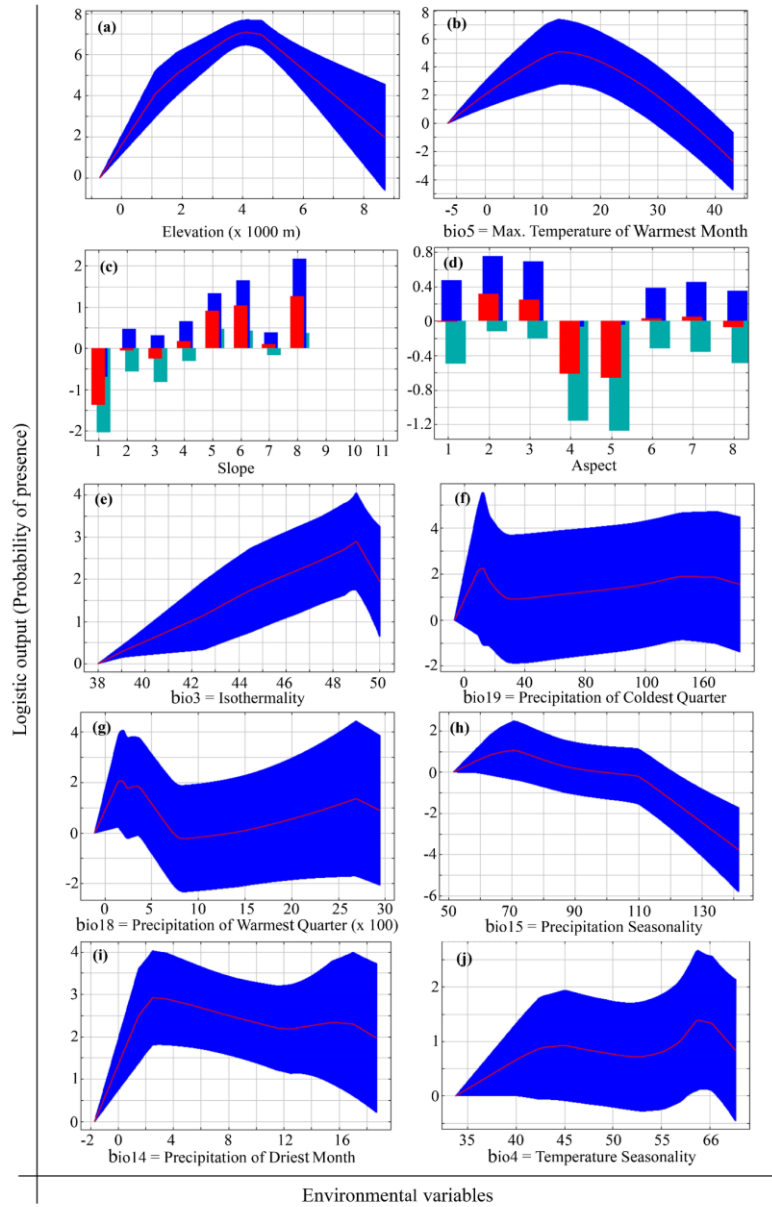


Figure 3: Response curves for predictor variables employed in logistic model used to estimate likelihood of *Neopicrorhiza scrophulariiflora*'s occurrence. Panels show (a) Elevation, (b.) bio5 = Maximum temperature of warmest month, (c) Slope, (d) Aspect (e.) bio3 = Isothermality, and (f.) bio19 = Precipitation of coldest quarter, (g.) bio18 = Precipitation of warmest quarter, (h.) bio15 = Precipitation seasonality, (i.) bio14 = Temperature of driest month, (j.) bio4 = Temperature seasonality. Temperatures are measured in °C (degree Celsius) and precipitation in mm (millimeters); Aspects are represented by 1 (North), 2 (North–East), 3 (East), 4 (South–East), 5 (South), 6 (South–West), 7 (West), 8 (North–West). The slope is represented by 1 (-1° – 3°), 2 (3° – 8°), 3 (8° – 13°), 4 (13° – 18°), 5 (18° – 23°), 6 (23° – 28°), 7 (28° – 33°), 8 (33° – 38°), 9 (38° – 43°), 10 (43° – 48°), and 11 (48° – 53°).

The habitat suitability was also influenced by other temperature or precipitation variables, but their contributions to the model were relatively low (bio18: 2.9%, bio15: 2.7%, bio14: 1.5%, bio4: 0.5%). Jackknife tests of the importance of different variables

showed that elevation, bio5, and bio18 (precipitation of warmest quarter) had the highest training contributions (>0.3) when used in isolation (**Figure C.1**).

2.4.2 Current Potential Habitat Suitability

The estimated area, potentially suitable for *Neopicrorhiza scrophulariiflora* under recent climatic conditions (1970–2000) was 11,616.5 km² (ca. 7.9% of the total area of Nepal), with suitable habitats confined to 28 districts in the high mountains of Nepal (**Figure 4, Table 3**). Dolpa district in western Nepal had the highest suitable area of 1436.0 km², corresponding to ~1.0% of the total area of the country, followed by Taplejung in the east (1256.8 km²; 0.9%). Most of the suitable area was located in three districts, namely Manang in the central part of Nepal (34.6%), and Taplejung (34.5%) and Sankhuwasabha (29.5%) in the east.

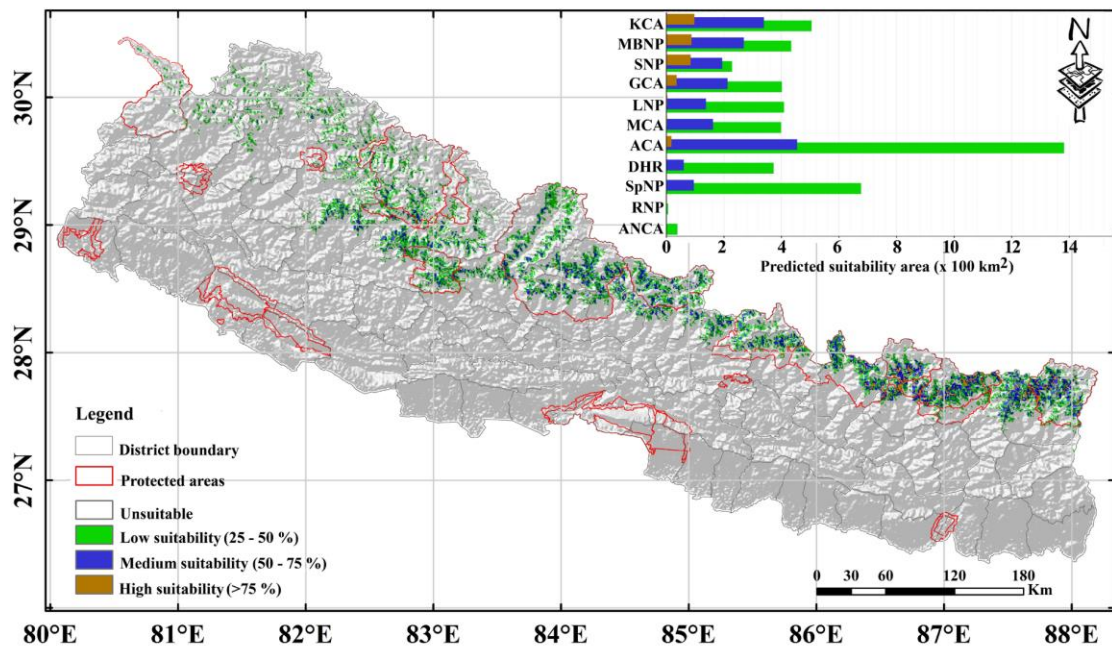


Figure 4: *Neopicrorhiza scrophulariiflora* habitat suitability in the Nepalese Himalaya, including protected areas, depicted in the graph. Suitability is displayed in three different levels: low (25–50%), medium (50–75%), and high (>75%). Protected areas with suitable habitat areas are Kanchenjunga Conservation Area (KCA); Makalu Barun National Park (MBNP); Sagarmatha National Park (SNP); Gaurishankar Conservation Area (GCA); Langtang National Park (LNP); Manaslu Conservation Area (MCA); Annapurna Conservation Area (ACA); Dhorpatan Hunting Reserves (DHR); Shey-Phoksundo National Park (SpNP); Rara National Park (RNP); Api-Nampa Conservation Area (ANCA)

Table 3: *Neopicrorhiza scrophulariiflora* habitat area across districts in the Nepalese Himalaya, according to estimates

District	Low (km ²)	Medium (km ²)	High (km ²)	Total suitability in district (km ²)	% of suitable area in district	% of suitable area in country
Western Nepal						
Baglung ^a	115.96	25.60	0.00	141.56	7.94	0.10
Bajhang ^{ab}	140.06	3.01	0.00	143.07	4.18	0.10
Bajura ^a	110.69	3.76	0.00	114.46	5.23	0.08
Darchula ^{ab}	279.36	153.61	13.55	446.53	16.81	0.30
Dolpa ^{ab}	1196.51	239.45	0.00	1435.97	18.20	0.98
Humla ^{ab}	507.52	19.58	0.00	527.10	9.32	0.36
Jajarkot ^a	119.73	36.90	0.00	156.62	7.02	0.11
Jumla ^{ab}	378.76	42.92	0.00	421.68	16.66	0.29
Kalikot ^a	14.31	0.00	0.00	14.31	0.82	0.01
Mugu ^a	342.61	24.85	0.75	368.22	10.42	0.25
Rolpa ^a	0.75	0.00	0.00	0.75	0.04	0.0005
Rukum East ^{ab}	262.04	45.18	0.00	307.22	26.46	0.21
Rukum West	54.22	21.84	0.00	76.05	6.27	0.05
Central Nepal						
Dadhing	100.15	32.38	0.00	132.53	6.88	0.09
Dolakha ^{ab}	279.36	153.61	13.55	446.53	20.38	0.30
Gorkha ^{ab}	578.30	219.12	2.26	799.68	22.15	0.54
Kaski ^b	177.71	67.77	3.76	249.24	12.36	0.17
Lamjung ^{ab}	164.91	58.73	0.00	223.64	13.22	0.15
Manang ^b	511.29	251.50	13.55	776.34	34.57	0.53
Mustang ^b	647.58	137.80	0.75	786.13	22.00	0.53
Myagdi ^{ab}	341.86	71.53	3.01	416.41	18.13	0.28
Ramechhap	69.28	39.16	20.33	128.76	8.33	0.09
Rasuwa ^{ab}	304.21	93.37	0.75	398.34	25.80	0.27
Sindhupalchok ^{ab}	237.19	82.83	2.26	322.28	12.68	0.22
Eastern Nepal						
Panchthar	5.27	0.75	0.00	6.02	0.49	0.00
Sankhuwasabha ^b	615.95	331.32	79.82	1027.09	29.51	0.70
Solukhumbu ^b	406.62	329.81	125.75	862.18	26.03	0.59
Taplejung ^b	697.28	439.00	120.48	1256.75	34.47	0.85

Based on data provided by the Government of Nepal (GoN 2012), district area is computed. Symbols: 'a' indicates the presence of trade, while 'b' indicates species' availability (herbarium records) in those districts.

The central region (all districts of central Nepal) had the largest suitable area (5351.6 km², 3.6%), followed by the eastern (3218.3 km², 2.2%) and the western

(3046.6 km², 2.1%) regions (**Table B.4**). Due to the variation in the width (145–241 km) of the Himalayan range within Nepal in combination with the variation in precipitation, the potential habitat area was observed to widen from east to west, whereas the suitability decreased and the distribution of suitable areas became more fragmented towards the west (**Figure 4**). Highly suitable habitats covered a total of 386.3 km², most of which (87.1%) was concentrated in eastern Nepal. Areas of medium suitability covered about 2787.6 km², predominantly in eastern and central regions, and areas of low suitability covered 8442.6 km², which were distributed from east to west in the higher mountains (**Table 3, Figure 4**).

There are eleven protected areas (PAs) in the high mountains. Highly suitable habitat areas of *N. scrophulariiflora* were located within five of these PAs spanning across 14 districts. From east to west these were: Kanchenjunga Conservation Area (KCA), Makalu Barun National Park (MBNP), Sagarmatha National Park (SNP), Gaurishankar Conservation Area (GCA), and Annapurna Conservation Area (ACA) (**Figure 4**). Nine PAs spanning across 13 districts included areas of medium suitability. In addition to the five already mentioned PAs, these were Langtang National Park (LNP), Manaslu Conservation Area (MCA), Dhorpatan Hunting Reserves (DHR), and Shey-Phoksundo National Park (SPNP), mainly located in the eastern and central highland districts of Nepal. Furthermore, areas of low suitability occurred in two additional PAs, namely Rara National Park (RNP) and Api-Nampa Conservation Area (ANCA) spanning across three districts of western Nepal (**Figure 4, Table B.5**). Protected areas covered about 61.2% (7108.3 km²) of the total estimated area of suitable habitats. The remaining part of the projected distribution was located outside protected areas (**Figure 4, Table B.6**). Comparing the estimated habitat suitability within different PAs, KCA had the largest area (97.1 km²) categorized as highly suitable and ACA had the largest overall area (1854.6 km²) of suitable habitats. RNP only included areas of low suitability and the predicted habitat area (6 km²) was lower than for any other PAs, presumably due to its small size (106 km²) and location at low elevation (**Table B.6, Figure 4**).

2.4.2 Trade System and Composition

Neopicrorhiza scrophulariiflora was traded from 20 districts (13 with and 7 without recorded occurrences of the species based on the herbarium survey), mostly in western Nepal (**Figure 5a, Table 3**). The total traded volume for the 12 fiscal years, 2004/05 –

2015/16, was 372 tons. There was no significant correlation between the traded volume (12 years) for districts and the predicted distribution of areas of high and medium habitat suitability. Also, a positive relationship ($r = 0.642$, $p = 0.002$) between the traded volume and areas of low suitability indicates that the resource exploitation patterns did not align well with the predicted occurrence of *N. scrophulariiflora* populations. At the same time, annual trade frequency across the 12 fiscal years (2004/05 to 2015/16) showed positive association with areas of low ($r = 0.604$, $p = 0.005$) and medium ($r = 0.473$, $p = 0.035$) suitability but there was no significant association with the area of high suitability. These patterns seem to indicate that exploitation was mainly high in areas with limited resources.

The average annual trade for the 12-year period was 31 tons, varying between a minimum of 10.3 tons (2012/13) and a maximum of 59.9 tons (2015/16), and showed an overall increasing trend (**Figure 5b**). The frequency of reported trade was highest for Dolpa (11 out of 12 fiscal years), followed by Lamjung (8 out of 12 fiscal years). The average annual traded volume was highest in Humla (20.6 tons), followed by Dolpa (6.9 tons). The total traded volume over the 12 fiscal years was highest in Dolpa (75.0 tons), followed by Rukum East and Rukum West (62.0 tons, formerly Rukum District), and the least volume (<1 ton) was traded from Surkhet and Dailekh Districts (**Figure 5a**).

Western Nepal contributed 28.7 tons (92.7%) of the annual traded volume, central Nepal contributed 2.3 tons (7.3%) and, surprisingly, no trade was recorded from eastern Nepal. Nine out of 10 districts of the Karnali Province (Humla, Mugu, Dolpa, Jumla, Kalikot, Dailekh, Jajarkot, Rukum-west and Surkhet) contributed substantially with an average annual trade of 22.9 tons (73.8% of total), showing the dominance of Karnali in the trade of *N. scrophulariiflora* in Nepal. However, among the top six districts in terms of suitable habitat area (Dolpa, Taplejung, Sankhuwasabha, Solukhumbu, Gorkha, and Mustang) only Dolpa is located in the Karnali Province (**Table 3**).

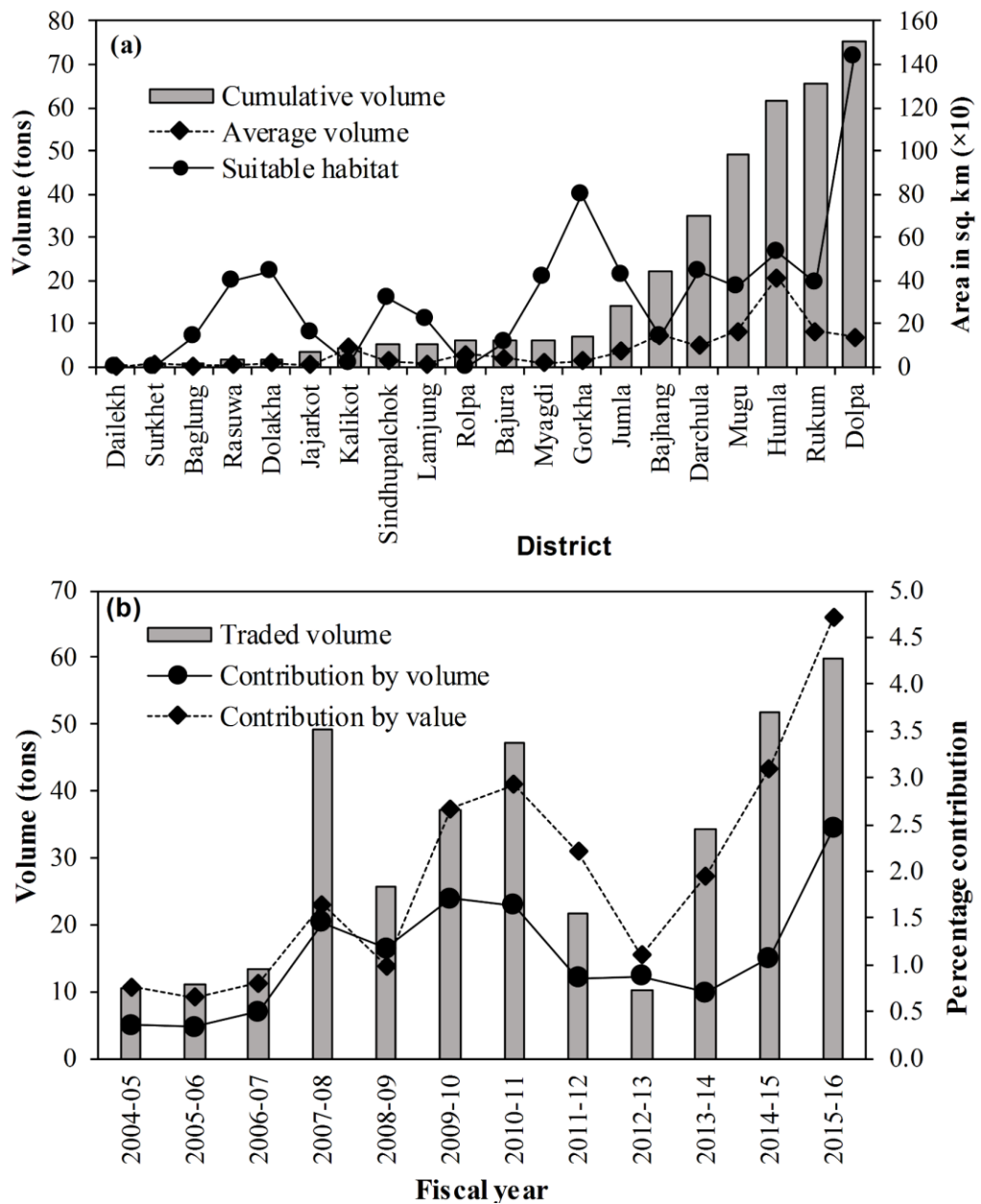


Figure 5: *Neopicrorhiza scrophulariiflora* trade in the Nepalese Himalaya: (a) cumulative volume traded from fiscal years 2004/05 to 2015/16, district-level area of suitable habitat, and mean annual traded volume; (b) volume traded from fiscal years 2004/05 to 2015/16, and % of the studied species by volume and revenue (value) to overall trade of medicinal plants. The model predicted the location of a suitable habitat (cf. Figure 3). The Department of Forest, Government of Nepal, provided the trade statistics.

2.4.3 Revenue Generation, Market-Trend and Profit Analysis

We compared the prices of *Neopicrorhiza scrophulariiflora* recorded from 2012 to 2016 at six trade stations: Nepalgunj and Kathmandu in Nepal, and Tanakpur, Delhi, Lucknow, and Kolkata in India. The unit prices were considerably lower in Nepal (mean ± SE: 10.8 ± 3.81 USD/kg) than in Indian markets (16.3 ± 2.88 USD/kg), likely due to factors such as transportation, storage, intergovernmental custom charges, rent-

seeking, and central wholesaler's margins (**Figure 6**). The average price in Nepal increased from USD 6.3/kg in 2012 to USD 17.1/kg in 2014 but decreased again to USD 9.4/kg in 2016. Over the same period, prices in India were higher but otherwise followed a similar trend, increasing from USD 9.8 to USD 28.4 and then decreasing to USD 12.8/kg. The average price difference between trade stations in Nepal and India was thus little more than 50% in 2012–2016. The average annual values of domestic trade and export (to India) of *N. scrophulariiflora* were USD 406,638 and USD 591,570, respectively (**Figure C.2**).

Between 2004/05 and 2015/16, *N. scrophulariiflora* contributed up to 5% (mean \pm SE = $1.96 \pm 0.34\%$) of the total annual government revenue on MAPs and up to 3% (mean \pm SE = $1.09 \pm 0.17\%$) of the total traded volume (**Figure 5b**). The average annual revenue collected from the *N. scrophulariiflora* trade was USD 4997 with a minimum of USD 1322 in 2012/13, and a maximum of USD 9479 in 2010/11.

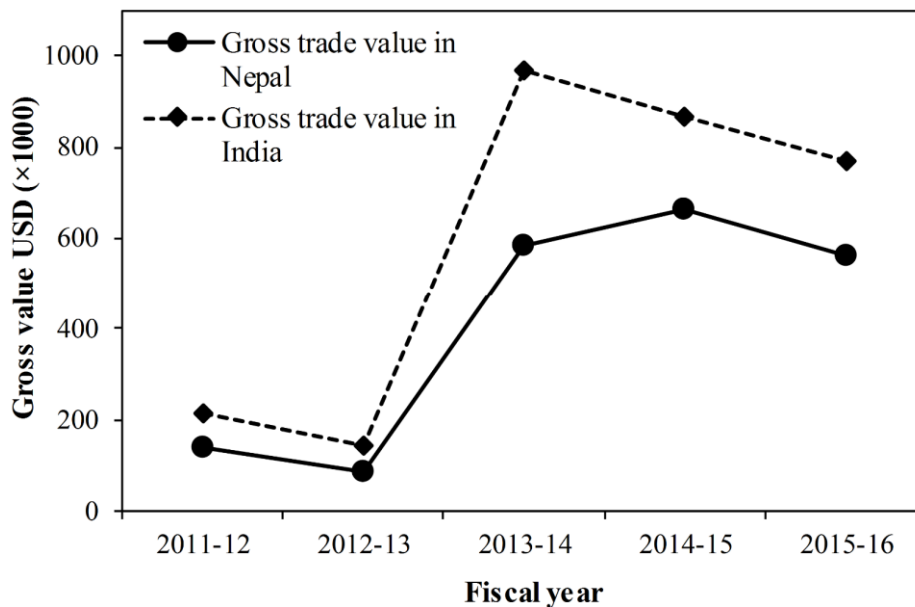


Figure 6: Gross sales of *Neopicrorhiza scrophulariiflora* in India and Nepal during the course of the previous five fiscal years, 2011/12–2015/16 (Source: Government of Nepal's Department of Forest)

2.5 Discussion

2.5.1 Environmental Factors Determining Habitat Suitability

Elevation was the most important factor used for predicting the distribution of *Neopicrorhiza scrophulariiflora*. Our results showed that the species had a very narrow optimal range (4000–4400 m) of occurrence and habitat niche. The decrease in projected habitat suitability on either side of this range might be related to a negative

effect of increasing solar irradiance towards higher elevations, possibly leading to UV damaging of the photosynthetic apparatus and assimilatory reduction; and temperature-dependent constraints towards lower elevations, possibly related to accelerated stomatal conductance, high evapotranspiration, and dehydration (Körner, 2003; Lütz and Seidlitz, 2012). In high alpine areas, low temperature, strong wind and desiccation, and a short growing season are the major constraints for plant growth (Körner, 2003; Poudeyal and Ghimire, 2011; Chapagain *et al.* 2019; Applequist *et al.*, 2020). The increasing temperature in the growing season (July/August) appeared to enhance the habitat suitability, nonetheless, areas warmer than 13°C decreased suitability. The specific combination of rainfall and temperature at the peak growing season might have a significant input in activating photosynthetic enzymes, thereby increasing the net photosynthesis per unit area and, hence, facilitating the node and leaf development (Larcher, 2012). Towards elevations lower than the optimal range, the plant might experience physiological constraints. For example, plants may experienced increased photo-respiration relative to carbon assimilation as the higher temperature rises the solubility of oxygen and reduces the concentration of photosynthetic (carbon acceptor) enzymes (Körner, 2003; Larcher, 2012).

In addition to the elevation, topographic variables like aspect (in general north/north-east slopes of the mountains) and slopes were found to determine habitat suitability (Figure 2a, 2c, and 2d). In agreement with this, previous studies (Ghimire *et al.*, 2005; Shrestha and Jha, 2009) reported that *N. scrophulariiflora* populations were generally found on the northern slopes and that their existence depended on the annual melting of snow. Other studies in the Himalaya suggest that south- and west-facing slopes receive particularly high solar radiation for longer diurnal periods and are thus considered to be comparatively hot and dry (Chhetri *et al.*, 2017; Panda *et al.*, 2017). The slope of the terrain (13–28° and 33–38°, Figure 2b) showed a positive correlation with the occurrence of *N. scrophulariiflora* indicating that the most suitable habitats exist neither in flat nor in too steep areas. Increasing steepness reduces snow cover, which reduces water percolation in the soil. Conversely, on flat land, more snow accumulates and remains for a longer time in spring, thus reducing the number of growing days (Larcher, 2012). On northern slopes, slower melting rates could provide moisture for a longer period of the growing season.

2.5.2 Regions for Predicted Distribution and Potential Cultivation

The MaxEnt ecological niche model showed that highly suitable habitats for *Neopicrorhiza scrophulariiflora* cover a limited area (~0.3% habitats being optimal in the total area of country) of subalpine–alpine zones of central and eastern Nepal. We found higher predicted habitat suitability in the eastern region of Nepal than in regions further towards the west. In agreement with this, the overall suitable area decreases towards the western region where suitable patches became more scattered and the overall distribution range widened as compared to the central and eastern regions. The western part of the country receives low annual (overall) precipitation and experiences the frequent number of droughts in a year, even though overall winter rainfall is slightly greater than in the eastern and central regions (Miehe *et al.*, 2001; Böhner *et al.*, 2015; Chhetri *et al.*, 2017).

N. scrophulariiflora is considered as moisture demanding species and needs adequate precipitation throughout the year for its perennial growth (Ghimire *et al.*, 2005; Poudeyal *et al.*, 2019). The precipitation in the coldest (winter) season (bio19) and in the warmest season (bio18, June/July: growing season) appeared as strong precipitation-dependent predictors of habitat suitability but was slightly weaker than the temperature-dependent predictors (**Figure 2**). These are comparable with Poudel *et al.*'s (2012) findings for *Taxus contorta* Griff. which showed that the distribution of this species is shaped by winter rainfall. In Nepal, winter rainfall enters from the western region, but it decreased when it moves towards the eastern region. Nevertheless, the central and eastern regions (specifically at elevations >2000 m) receive heavier and longer summer monsoon rainfall and experience a relatively smaller number of annual temperature extremes compared to the western region (Poudel *et al.*, 2012; Böhner *et al.*, 2015). The distribution of alpine plant species in Nepal Himalaya is widely influenced by the mean annual temperature and precipitation in the dry winter and growing (summer) seasons (Rana *et al.*, 2020; Kunwar *et al.*, 2020).

The higher values of projected habitat suitability in the eastern region suggest that this region might be suitable for cultivation and gene pool conservation of *N. scrophulariiflora*. Meanwhile, in the western region, Dolpa and Humla have widespread habitat potential, indicating that these districts have relatively high winter precipitation compared to that of other western districts of Nepal (Böhner *et al.*, 2015). Nevertheless, highly suitable areas are not predicted to exist in these districts of western

Nepal, which may be due to comparatively higher temperature than the optimum level. Future assessments incorporating a higher number of abiotic (such as soil nutrients) and biotic factors (disturbances) might help to improve prediction of the habitat suitability in these areas. This may help to identify areas in these districts that can be employed in cultivation trials of *N. scrophulariiflora*, an option that appears, even more, promising in almost all eastern districts (**Table 3**) and Gorkha and Mustang in Central region compared to the neighboring districts.

2.5.3 Asymmetric Trade Structure

Our analysis provided that the volume of *Neopicrorhiza scrophulariiflora* rhizomes exported in fiscal year 2015/16 was six times greater than in 2004/05. The revenue generation was also increased almost six times between those two fiscal years. When dealing with national consumption, volumes of *N. scrophulariiflora* received by domestic industries in Nepal is limitedly available. Tiwari *et al.* (2004) reported that 750 kg (price rate 1.6 USD/kg) of dried rhizomes of *N. scrophulariiflora* was used in fiscal year 2003/04 by the Kathmandu-based business companies. A recent study by Kafle *et al.* (2018) revealed that increased private investments on herbal industries have accelerated the domestic market consumption, which has increased almost three times (~2000 kg/year, price rate 11.2–15.5 USD/kg) to that previous record for the Kathmandu valley, and overall Nepalese industries consumed a total volume of 6076 kg. However, domestic consumption just represented 10 times lesser than the overall export volume. Ghimire *et al.* (2016) found that the export value of traded MAPs, including *N. scrophulariiflora*, increased from USD 27 million in 2005 to USD 60 million in 2014 due to increased unit cost prices. Also, their evidence pointed out that resource exploitation is more confined to the limited number of high-valued MAPs, such as *N. scrophulariiflora*. The escalating trade demand and increasing prices can be linked with the economic growth of the neighboring countries, India and China, having high purchasing power (Pyakurel *et al.*, 2018).

Aside from that, the annually traded volume of 31 tons of *N. scrophulariiflora* from 20 districts, based on DoF data, appears to be far lower than the actual traded quantity. Pyakurel *et al.* (2018) recorded the trade of almost 19 tons from Darchula district alone. Darchula only covers total suitability of 446.5 km² (nearly 4% of the country) whereas all other districts in Nepal have 11547.2 km² (96 % of the total suitable area). This indicates that *N. scrophulariiflora* is likely available in other

districts in much larger quantities. It can be assumed that *N. scrophulariiflora*, with high international demand and assumed wider availability in Nepal, is traded in far higher quantity than what is registered in government data. Pyakurel *et al.* (2018; 2019) documented the trade of government banned medicinal plants, showing that products in high demand are traded. Olsen (2005) recorded the trade of 529 tons from Nepal in 1997/98, further strengthening the assumption. There are multiple reasons for the limited documentation of trade amount by DoF; (1) *N. scrophulariiflora* is distributed in high-mountain districts, mostly in protected areas, and DNPWC, rather than DoF have the management authority in protected areas, and our analysis is based on DoF data, (2) reluctance of divisional forest office (former district forest office) personnel to forward the trade data to DoF as they are not offered any economic incentives to do so (Pyakurel, 2020), (3) traders often underreport the traded volume to save royalty or to conceal volume over the volume that was legally permitted by the authorities. Such types of underestimation have been observed elsewhere in Nepal (Olsen, 2005; Devkota *et al.*, 2017; Pyakurel *et al.*, 2018).

Officially traded quantity and availability of medicinal plants are not often correlated as multiple factors determine the trade. There are no official records of the trade from eastern Nepal even though the three Himalayan districts (Taplejung, Sankhuwasabha, and Solukhumbu) in this region have substantial areas of high suitability (**Table 3**). The lack of official trade is surely due to (1) ban of collection of forest products from national parks by law and MBNP and SNP are both located in the highlands of Sankhuwasabha and Solukhumbu districts where the suitable areas are found, (2) KCA in the highlands of Taplejung has its management unit and data are not transferred to DoF. This does not rule out the possibility of the trade from eastern Nepal though. Ghimire (2009) mentioned the centuries-old trade history of medicinal plants trade from Walangchung Gola of eastern Nepal to Tibet and reported an annual trade of six tons *N. scrophulariiflora* rhizomes released from Taplejung district between 1996 to 2004. Likewise, Pyakurel (2008) reported the undocumented trade of 10–12 tons of fresh plant material from Taplejung to Tibet.

The trade from western Nepal is more organized because of a centuries-old trade system relative to eastern Nepal. Nepalgunj has been a MAP trading hub for centuries and most of the exporters operate from this town. As a result, a trust-based trade network, rather than a formal and legitimate system, is established and connects town, villages, and alpine meadows and enables the export of huge quantities of MAPs

(Hamilton, 2004; Pyakurel *et al.*, 2018). Similarly, the dependency on collection and trade of medicinal plants, including *N. scrophulariiflora*, for cash income in western mountain regions could be another important reason for higher trade from western Nepal compared to the eastern region (Hamilton, 2004; Kunwar *et al.*, 2013; Devkota *et al.*, 2017).

Official records from DoF showed that *N. scrophulariiflora* is traded from 20 districts. These 20 districts include districts like Surkhet where plant species is not available; or Panchthar, Rolpa, Kalikot with very limited suitable areas (**Table 3**). Local traders transport MAPs based on a transport permit, which is valid for 21 days and is issued by the district of origin. In some cases e.g., natural calamities, traders have to store MAPs for prolonged periods *en route*, and the transport permit expires. In such a case, traders receive a new permit from another district, resulting in the documentation of trade from districts where MAPs are not available.

2.6 Conclusion and Future Recommendation

We found about 8% of the area of Nepal, located in the high mountains, is potentially suitable as habitat for *Neopicrorhiza scrophulariiflora* but areas of high suitability (<1%) are limited to a narrow zone of elevation (4000–4400 m). Due to higher annual rainfall, a significant proportion of the optimal habitats are located in the eastern mountains, but areas in other parts of the country could be used for the development of organized cultivation. The trade of the plant represents a substantial economic contribution (up to 5% revenue) to the national herbal market and thereby also provides cash income to rural communities, particularly in the western Nepal. Nevertheless, the concentration of the most suitable areas in the eastern region in combination with the increasing trade from the western region, which has limited suitable areas, indicates that the prevailing resource exploitation is likely to be unsustainable. It supports the hypothesis that overexploitation and high trade in the areas with climatically less suitable for the growth might cause the extinction of the local populations of *N. scrophuriiflora* in near future, which is indeed worrisome for western Nepal. Praiseworthy to state that, Dolpa and Mugu districts in western region receive relatively high winter rainfall and could be considered for experimentation with cultivation practices to supplement the natural resource base.

The potential distribution map that we developed in this study could be used as baseline information when setting up management policies for sustainable conservation

of threatened habitats. We understand that our estimated suitability areas are based on niche modeling and largely determined by climatic factors. Nevertheless, we suggest more rigorous empirical assessments of other possible *N. scrophulariiflora* locations. There may be factors that determine the actual distribution other than climatic factors, demanding the empirical resource assessment data to quantify the harvestable amount. Comparing the empirical resource assessment data with modeled data will give a clear pathway for the future, which one to choose among the two. The present analysis indicates that the eastern and central mountains should have considerable potential for harvesting the plant. However, caution is essential, and we, therefore, suggest conducting a rigorous empirical study on the harvest response of plant populations as a basis for identifying the optimal annual harvest and developing sustainable management strategies.

CHAPTER 3

3. ENVIRONMENTAL EFFECTS ON DENSITY AND BIOMASS¹

3.1 Abstract

A surprisingly large number of species potentially threatened by human harvest lack quantitative ecological studies incorporating harvest effects, especially clonal species in the alpine Himalayas. We studied density and biomass variation of a threatened medicinal herb, *Neopicrorhiza scrophulariiflora*, to examine the effect of harvest on plant performance. The study covered two regions with contrasting harvest situations – one with open-access and another protected from commercial harvesting. Four populations from each region were compared along an elevation gradient (3800–4800 m). Also, we conducted *in situ* interviews with 165 and 38 medicinal and aromatic plant users in open-access and protected regions, respectively, to assess the collection and use patterns of the target species. The quantity harvested per household for traditional healthcare use was similar in both regions. We found no evidence of trade-driven collection in the protected region but in the open-access region a trade-based annual collection of 35–465 kg dried rhizomes per household had a strong negative effect on both density and biomass. In the protected region, the effect of harvest intensity on plant density was positive for vegetative and negative for reproductive individuals, whereas in the open-access region, the effect was negative for both vegetative and reproductive individuals. The results indicated that a low harvest intensity had no adverse impact on *N. scrophulariiflora* populations; however, quantification of the optimum level of harvest remains to be explored. Shrub vegetation appeared to buffer the harvest impact on plant density, possibly through the retention of additional moisture. To maintain population viability, we suggest regulating harvest, for example by introducing rotational harvest systems, ensuring that a sufficient number of reproductive individuals are left as a source of propagules in each harvested population and that populations are given time to recover between harvests.

Keywords: Clonal herb, medicinal plant, trade, elevation gradient, shrub facilitation, conservation

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3.2 Introduction

Plant life in high mountains, such as the Himalayas, is constrained by biotic and abiotic stress. Contemporary ecological studies from alpine regions primarily emphasize extreme abiotic conditions where plant growth is constrained by short growing season, low temperature and limited resources (Klimešová *et al.*, 2012; Dvorský *et al.*, 2015). Interactions with biotic factors, including herbivory, habitat competition and facilitation, and anthropogenic activities are issues that are not well addressed in plant population studies from alpine areas in the Himalayas (but see Chu *et al.*, 2008; 2009; Ghimire *et al.*, 2008a) and elsewhere (Callaghan *et al.*, 1992; Körner, 2003). Alpine plant species in the Himalayas are mostly perennial and many of them exhibit clonal growth (Klimeš, 2003; Klimešová *et al.*, 2011). Clonal units in such species not only share resources and photosynthates but also absorb negative effects of physical stress, competition, and herbivory, enabling alpine plants to respond to environmental changes (Lei, 2010; Klimešová *et al.*, 2011). At habitat level, the inter-species competition is overlaid by the facilitative role of surrounding vegetation (Wang *et al.*, 2014; Ballantyne and Pickering, 2015). Delivery of shade and shelter, moisture retention, nutrient accumulation, temperature regulation, and defense against herbivory and physical damage are important services that can be offered by the surrounding vegetation (Callaway *et al.*, 2002; Ballantyne and Pickering, 2015). Thus, the balance between facilitative and competitive environmental interactions has significant ecological implications to the performance of alpine plant species (Chu *et al.*, 2009; Klimešová *et al.*, 2012).

In addition to environmental interactions, alpine plants are in many cases also subjected to high levels of anthropogenic impacts. In the Himalayas, for example, international trade in medicinal and aromatic plants (MAPs) (Olsen, 2005; Pyakurel *et al.*, 2018) has created extreme pressure on populations of some alpine plants and their habitats. In addition, other factors, such as livestock grazing and fire, may reduce densities of alpine plant populations (Niu *et al.*, 2016). Harvesting of whole plants or plant parts may affect reproduction, survival, and growth, thereby affecting plant population dynamics (Ticktin, 2004; Ghimire *et al.*, 2005; 2008; Gaoue *et al.*, 2013; Huai *et al.*, 2013). The extent of harvest impact on plant populations, however, varies depending on habitat conditions, plant growth strategies, and species-specific

processes, such as regeneration and colonization (Ticktin, 2004; 2015; Gaoue *et al.*, 2013). Clonal plant species to some extent buffer the anthropogenic effects like harvesting by mobilizing stored reserves to maintain normal phases of growth (Mandle and Ticktin, 2012). Furthermore, the clonal tendency towards developing a bud bank after a disturbance also plays a crucial role in vegetative reproduction (Evette *et al.*, 2009). The clonal architecture, especially in plants exhibiting a 'guerilla' strategy of clonal growth, producing long and spreading vegetative offshoots, allows the plant to escape stressful microsites and colonize more suitable ones (Humphrey and Pyke, 1998; Ghimire *et al.*, 2005). In clonal herbs exhibiting a 'guerilla' strategy the density of plants is positively affected by light harvesting if it occurs at sufficiently long intervals and does not exceed the regeneration potential (Ghimire *et al.*, 2005). Thus, with responsible management practices, vegetative propagation in clonal plant species could compensate for reduced biomass caused by anthropogenic disturbances.

The interactions between people and plants in the Himalayas is not yet fully understood (but see Ghimire *et al.*, 2005; 2008; Rokaya *et al.*, 2017). Specifically, determining the relative effects of habitat factors, such as substrate, vegetation cover, and disturbance on plant density and biomass is crucial for development of well-informed and effective conservation policies. This study aims to disentangle the factors that control the density and biomass of a clonal medicinal herb, *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong, across a continuum of environmental conditions and harvest intensities in the alpine Himalayas, Nepal. This species is highly threatened due to overharvesting of its rhizome for regional and international trade (Ghimire *et al.*, 2005). Studies emphasizing the variation in density and biomass of such species across a continuum of environmental conditions along an elevation gradient could enhance our understanding of the ability of the species to respond to environmental stress. We will address three questions: (i) In what ways do plant utilization patterns vary between study regions in terms of the purpose and intensity of harvest, and whether harvest takes place in the beginning or at the end of the growing season? (ii) How do elevation, surface and vegetation cover, and anthropogenic disturbance influence the density and biomass of *N. scrophulariiflora*? and (iii) Does plant response to harvest impact vary between study regions? To answer these questions, we first assessed plant utilization systems and analyzed density, population structure, and biomass variation in different populations along elevation gradients in

two study areas characterized by different protection regimes. The species' interaction with environmental factors, including habitat conditions, surrounding vegetation, and human harvest regimes were analyzed to evaluate these associations.

3.3 Materials and Methods

3.3.1 Study Area

The study was conducted in two protected areas (hereafter referred to as regions): Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP) in the north-western and north-central parts of Nepalese Himalayas (**Figure 7**). Both protected areas in those regions are managed by the Department of National Parks and Wildlife Conservation of the Government of Nepal. The local Community-led Management Council for ANCA (located in Darchula District) and the national park office for LNP (in Rasuwa District) are the management bodies responsible at the local level. The climate in both regions varies from subtropical to alpine-nival. The two regions are similar in terms of annual precipitation (average of 2100 mm in ANCA, and 2300 mm in LNP) and annual average temperature (range 4–27°C for ANCA and 2–25°C for LNP; both precipitation and temperature were recorded at the nearest meteorological stations located at 1900 and 1100 m above sea level, respectively). Both regions are characterized by high mountains and rugged terrain. Almost 70% of residents in ANCA and about 50% in LNP live below the poverty line (Uprety *et al.*, 2011; Pyakurel *et al.*, 2018). Local livelihoods depend on traditional agriculture, livestock rearing and seasonal labour (CBS, 2012). The upper slopes are fragile and arable fields are limited to low elevations (<3000 m), but the productivity is lower in ANCA than in LNP (Fox *et al.*, 1996; CBS, 2012; DDC, 2015). Therefore, people in ANCA supplement their income mostly by harvesting and selling high-valued MAPs and other forest products (Pouliot *et al.*, 2018; Pyakurel *et al.*, 2018). In LNP, people primarily supplement their income through livestock rearing, cheese production, and tourism, and are less involved in harvesting and trade of forest products (Shakya, 2016).

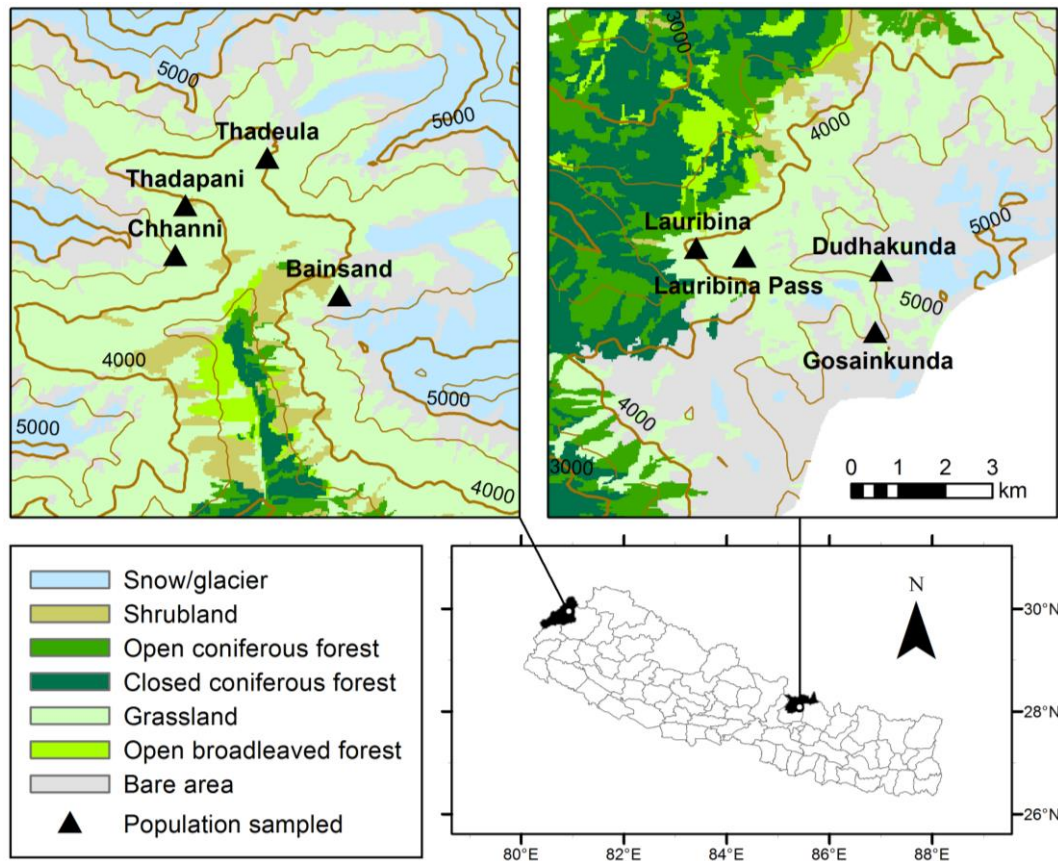


Figure 7: Study area map showing populations (indicated by triangles) examined at Langtang National Park (right) and Api-Nampa Conservation Area (left) in Himalaya Nepal. The elevation scale is defined by the thin and thick contour lines at intervals of 500 and 1000 m from 3000 to 5000 m (Source: Uddin *et al.*, 2015).

ANCA was recently established in 2010, and in this region commercial harvesting of MAP is legally permitted. The ANCA Management Council issues collection and transportation permits for MAPs (GoN, 2015). LNP has been protected from commercial harvesting of forest products since its establishment in 1976, and to date, trade permissions have not been issued for any products, except for a high-value medicinal caterpillar fungus, *Ophiocordyceps* (*Ophiocordyceps sinensis* (Berk.) G. H. Sung *et al.*) (HMG/N, 1978; personal communication with the conservation officer, 26 July 2018). Owing to the differences in MAP harvest and trade regulations, we hereafter refer to ANCA as 'open' and LNP as 'protected'.

3.3.2 Study Species

Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong (hereinafter referred to as *Neopicrorhiza*) of the family Plantaginaceae (previously Scrophulariaceae) (APG IV,

2016) is a perennial, rhizomatous herb that exhibits clonal growth. It is confined to the pan-Himalayas, the Tibetan Plateau, Assam-Burma and south-central China, and is found at elevations between 3500–4800 m (Hong, 1998; Ghimire *et al.*, 2005). The plant is relatively slow growing and is mostly confined to cool north-facing slopes (Shrestha and Jha, 2009). Flowering and fruiting occur in June–September. Seedling recruitment is low, and propagation mainly takes place by vegetative means (Ghimire *et al.*, 2005). The modular growth of the individual genet takes place by horizontal extension of ramets (vegetative offshoots), which can survive independently when detached. The clonal growth of *Neopicrorhiza* has been described as a ‘guerrilla’ strategy, leading to spaced clusters of spreading fugitive ramets connected below ground by horizontal rhizomes (Ghimire *et al.*, 2005).

The rhizomes of *Neopicrorhiza* are valued for the treatment of cough, cold, headache, fever, high blood pressure, conjunctivitis, bile reflux, and intestinal pains, among others (Manandhar, 2002; Ghimire *et al.*, 2005). It is one of the most commonly consumed herbs in the Himalayas and is appraised to have a high pharmacological potential (Li *et al.*, 2014). The plant is resistant to grazing, and thus, premature and excessive harvesting is the main issue for its conservation (Ghimire *et al.*, 2005; Shrestha and Jha, 2009). It is closely related to *Picrorhiza kurrooa* Royle, a species native to North-west India listed in CITES Appendix II. Rhizomes of both species are traded globally, but *Neopicrorhiza* is more commonly traded than *P. kurrooa* (Mulliken, 2000). Rhizomes of *Neopicrorhiza* collected from Nepal are mostly traded to India in air-dried form through well-established marketing chains (Olsen, 2005) with a current annual trade volume of ca. 350 tons (personal communication with traders in ANCA and at a major road-head collection center in Nepalgunj, south-west Nepal, November/December 2017).

3.3.3 Interviews with Local Resource Users

This work was carried out as a partial basis for the first author’s Ph.D. dissertation at the Central Department of Botany (CDB), Tribhuvan University, Nepal. The study proposal was approved by the CDB Research Committee. Written permission for fieldwork was obtained from Department of National Park and Wildlife Conservation, Government of Nepal. The research objectives were explained, and prior informed consent was received from respondents before conducting interviews and consultations

with MAP users in ANCA and LNP. The participants were informed they could withdraw their consent at any point in case of disagreement.

Commercial MAP harvesting and livestock grazing are the main human activities in the alpine regions of the Himalayas. Local people usually follow traditional systems of rotational grazing when livestock are brought to subalpine and alpine pastures for summer grazing. We recorded two types of MAP collectors: dedicated collectors, who collect only one target species at a time and are not involved in pastoralism; and opportunistic collectors, who collect different MAP species and are also involved in pastoralism (Olsen, 1998). On average across the grazing seasons of 2015–2017, we recorded 1500 livestock (100 cattle and 1400 sheep/goats) and 30 herders in ANCA, and 1000 livestock (200 cattle and 800 sheep/goats) and 20 herders in LNP at the study locations (based on interviews with herders, 2015–2017). Commercial harvesting of *Neopicrorhiza* usually takes place during the late part of the growing season (August–October). Apart from this, in ANCA, harvesting of *Neopicrorhiza* usually coincides with the harvesting season of *Ophiocordyceps* in late May to early July. We used *in situ* open interviews with 165 MAP users in ANCA and 38 in LNP to solicit information about harvesting and utilization. We assessed purpose and level of harvest of *Neopicrorhiza* by asking four questions: (i) Do you harvest *Neopicrorhiza*? (ii) If yes, then for what purpose (only for domestic consumption or also for selling)? (iii) Which season do you prefer to go for collection? and (iv) How much amount do you collect typically in a year?

3.3.4 Plot Establishment and Sampling

We carried out a sample-based study in *Neopicrorhiza* populations during the peak growing season (June–August of 2015–2017). We identified four large populations of *Neopicrorhiza* in each of the two regions, within an elevation range of 3500–4800 m along upper Chameliya valley of ANCA and upper Trishuli watershed of LNP (**Figure 7, Table B.7**). In each population, we laid out three transects at intervals of 100 m (Ghimire *et al.*, 2005; 2008). In each transect, we established two plots (3 × 3 m) at horizontal distances of at least 20 m from each other, depending on field conditions. The clonal behavior of the study species is such that sprouting occurs readily from the parental ramet within a limited distance leading to a patchy form of distribution. Therefore, we established the plots subjectively where the plants occurred in sufficient

number for sampling (Whittaker, 1956). We divided each plot into nine subplots (1×1 m). Among these, five subplots (four at the corners and one at the centre) were systematically sampled and measured. In essence, the study included 48 plots with 240 subplots, and each of the samples in the two regions included half of these.

We studied plant density for ramet stage classes. In each subplot, we classified individual ramets, based on number and size of leaves and occurrence of reproductive organs, into one of the three stage classes (Oostermeijer *et al.*, 1996; Weppeler *et al.*, 2006): (i) juvenile ramets bearing 1–4 smaller-sized (range: 0.5–2.0 cm length) leaves; (ii) adult vegetative ramets bearing more than four larger-sized (>1.5 –9.0 cm length) leaves but with no indication of sexual reproduction; and (iii) reproductive ramets bearing any number and size of leaves and a reproductive peduncle. To describe population structure, we calculated the proportion of ramets in each stage class.

For the estimation of harvestable rhizome biomass, we asked local harvesters to collect 2–6 full-grown ramets (adult vegetative and reproductive stages only) from eight to eighteen 1×1 m plots in each population, laid out randomly in the vicinity of plots used in density estimation. This way we collected biomass samples from 50 subplots in ANCA and 46 in LNP. In total, we extracted 161 biomass samples in ANCA and 133 in LNP (30–59 samples including above and below-ground parts per population). We measured the length and girth of each rhizome when fresh. Girth was calculated as a mean of three measurements at different nodal points along the rhizome. The volume of each rhizome was calculated based on girth and length assuming cylindrical shape. The biomass of each rhizome sample was measured after drying for 72 hours at 60°C in a hot air oven. The volume and biomass were used for calculation of rhizome tissue density (expressed in g cm^{-3}). The total number of rhizomes per kilogram dry weight was calculated for each population based on the estimated mean biomass.

3.3.5 Plot-Based Environmental Variables

Geographical location (latitude and longitude) and topographic features (elevation, slope, and aspect) were measured for each plot. Latitude, slope, and aspect were used to calculate potential annual direct incident radiation (PADIR) (McCune and Keon, 2002; McCune, 2007). Vegetation data were recorded in each subplot (**Table B.8**). The shrub canopy cover was estimated visually as total canopy cover of all shrub species,

and shrub height was measured at three points located diagonally across the subplot. Cover of ground vegetation was estimated separately for herbs (all dicotyledons species, orchids, and lilies), graminoids (including all grasses and sedges), and lichens/mosses. Similarly, bare ground cover and rock cover were estimated for each subplot. Soil pH was measured using a portable pH meter (Mudder brand, United States of America, accuracy: ± 0.3) in each subplot as an average of three measurements taken diagonally at equidistant points.



Figure 8: *Neopicrorhiza scrophulariiflora* grows in both intensively harvested and unharvested environments; a single plant is between 5 and 25 cm tall while it is in the fruiting stage. The scale bars show the objects' approximate sizes

Evidence of MAP harvesting and livestock grazing were confirmed by intensive observation in each subplot. In order to determine the magnitude of impact, subplots were further divided into four cells, each 0.25 m^2 in size. We assigned a binary score to each cell based on whether there was evidence of disturbance (1) or not (0). At the subplot level, the scores given separately for each category of disturbance were combined for the four cells to obtain an overall score ranging from 0 (null) to 4 (very high). The following types of evidence were used in assessing the harvesting of *Neopicrorhiza*: the presence of left-over rhizome fragments, uprooted plants, and holes or scars left after the excavation of plants (**Figure 8**). Assessment of grazing was based

on the presence of pugmarks and trampling, bite marks, browsed plants, broken, defoliated or disorientated aerial parts and animal droppings.

3.3.6 Data Analysis

Variation in ramet density, biomass and environmental covariates among populations and between regions were tested by non-parametric Kruskal Wallis one-way ANOVA and Mann-Whitney U tests. A generalized linear mixed effects model (GLMM) was used to examine the effect of environmental factors on ramet density, and a linear mixed effects model (LMM) was used for analyzing rhizome biomass and tissue density. A negative binomial distribution was assumed for ramet density, and rhizome biomass and tissue density were transformed using the natural logarithm to make their residuals normally distributed. To control for intra-class correlation caused by the nested design, ‘plot’ was included as a random effect in models for ramet density. Similarly, a random effect of ‘subplot’ was included in models for rhizome biomass and tissue density. Effects of region and population were treated as fixed nominal factors. All numeric environmental predictors (**Table B.8**) were treated as covariates. These covariates were standardized (mean 0, standard deviation 1) to avoid erroneous conclusions when analyzing interactions between continuous variables and to facilitate the interpretation of variables and models (Bråthen and Lortie, 2016). We used qq-plots to evaluate the properties of residuals in models based on different distribution assumptions (Zuur *et al.*, 2009).

In the case of ramet density, the primary additive model was prepared by starting with a complete model, including all fixed effects and the random plot effect. Then, the model was reduced successively by backward elimination, gradually removing predictors that were not significant ($p \geq 0.05$). Based on the final model with only significant predictors, extended models including two-way interactions between regions, harvest and elevation were prepared. In the case of rhizome biomass and tissue density, the number of potential predictors was lower and a selection procedure was not applied. For each model, the Akaike Information Criterion (AIC) was calculated and compared with the null model. The model with the lowest AIC value was considered as the best model. To assess the validity of the selected model, we performed likelihood ratio tests comparing models with fixed effects to the null model. Pearson correlation analysis was used to examine co-variation between response and predictor variables

when more elaboration was required. The correlation analysis, Kruskal-Wallis and Mann-Whitney U tests were carried out in IBM SPSS 24 (IBM, 2016), and GLMM and LMM models were prepared using the lme4 package in R version 3.5 (R Core Team, 2017).

3.4 Results

3.4.1 Variation in Utilization Pattern

We recorded both opportunistic (26%) and dedicated (74%) collectors in ANCA. About, 5% of MAP users interviewed ($n = 165$) did not harvest *Neopicrorhiza*, 90% harvested it both for trade and local healthcare, and the rest 5% (all traditional healers) collected it only for local healthcare. Among the MAP users, 7%, 57%, and 31% collected *Neopicrorhiza* before the growth season (May/June), after the growth season (August/September) and both before and after, respectively. A conservative estimate of the annual collection is that on average people from ANCA collected 0.5–3.5 kg (mean \pm SE = 1.56 ± 0.06 kg) dry rhizomes per household for local healthcare and 35–465 kg (mean \pm SE = 170.50 ± 6.64 kg) per household for trade.

We found no evidence of trade-driven collection in the LNP region. In contrast to ANCA, all the MAP collectors interviewed in LNP were opportunistic, 87% (33 of the 38 interviewees) harvested *Neopicrorhiza*, and 88% of them told that the collection was for local healthcare. Only few users in LNP collected *Neopicrorhiza* during May/June (6%) and the majority collected in August/September (94%). Among the collectors, about 12% indicated willingness to sell the rhizomes should an opportunity arise, which is however unlikely due to the legal restrictions implied by the protected area system. Like in ANCA, people in LNP collected 1.0–3.0 kg (1.89 ± 0.09 kg) dried rhizome per household annually for local healthcare consumption.

3.4.2 Variation in Plant Density

Ramet density (m^{-2}) varied within and between the regions (**Figure 9**). Importantly, for total ramet density (all stages combined), LNP populations were 1.5 times denser than ANCA populations (mean \pm SE = 42.93 ± 3.35 for ANCA, 61.12 ± 3.83 for LNP, $p < 0.0001$). For individual stage classes, the densities in the two regions decreased in the same order from adult vegetative (33.60 ± 2.60 and 48.05 ± 3.07 , $p < 0.0001$), over juvenile (7.28 ± 0.66 and 10.03 ± 0.77 , $p = 0.002$) to reproductive (2.05 ± 0.31 and 3.03

± 0.37 , $p = 0.006$, in ANCA and LNP, respectively). In ANCA, only reproductive density varied significantly ($p = 0.004$) among populations and was highest in Thadeula and lowest in Thadapani (**Figure 9**). Similarly, in LNP juvenile density did not differ much among the populations, but adult vegetative density was highest in Lauribina and lowest in Dhudhakunda ($p = 0.002$), and reproductive density was highest in Gosainkunda and lowest in Lauribina ($p = 0.019$, **Figure 9**).

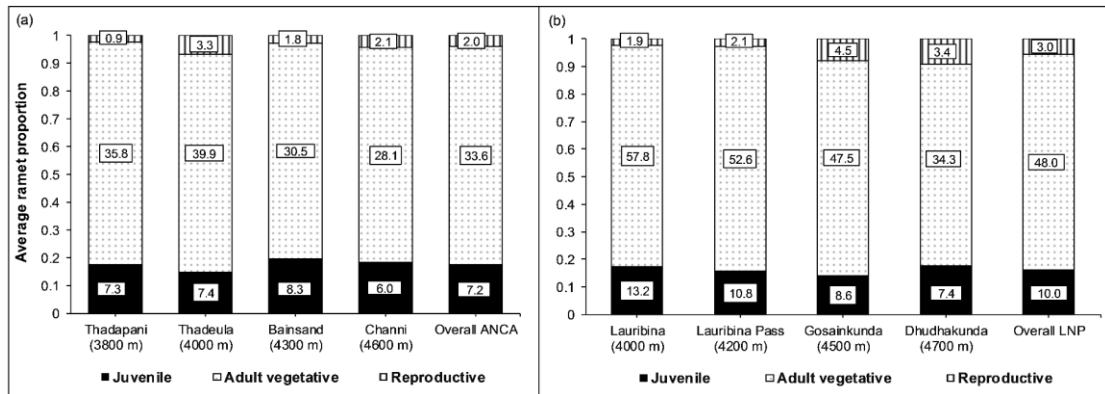


Figure 9. Population-based proportions of various life stages calculated using mean ramet density (m^{-2}) of *Neopicrorhiza scrophulariiflora* at two regions in Nepal: (a) Api-Nampa Conservation Area (left) and (b) Langtang National Park (right)

The population structure in terms of ramet stage class composition clearly deviated between the study regions ($p = 0.023$, Kolmogorov-Smirnov test) with greater reproductive ramet proportion in LNP (0.053 ± 0.006) than in ANCA (0.033 ± 0.004 , $p = 0.011$). By contrast, the proportions of ramets in other stage classes did not vary between the regions (**Figure 9**). Also, ramet composition did not vary substantially across populations within each region ($p = 0.267$ for ANCA and 0.656 for LNP).

3.4.3 Variation in Rhizome Biomass

Ramet girth (cm) was higher in ANCA (mean \pm SE = 0.211 ± 0.008) than in LNP (0.176 ± 0.006 , $p < 0.001$). Likewise, mean rhizome volume (cm^3) was also higher in ANCA (0.36 ± 0.05) than in LNP (0.26 ± 0.02 , $p = 0.023$). On the other hand, the rhizome tissue density ($g\ cm^{-3}$) was considerably higher in LNP (5.53 ± 0.37) than in ANCA (2.66 ± 0.22 , $p < 0.001$). In addition, the mean rhizome biomass (dry weight in g) was about 1.5 times higher in LNP (0.74 ± 0.03) than in ANCA (0.47 ± 0.02 , $p < 0.001$). In ANCA, mean rhizome biomass did not deviate between the populations (**Figure 10a**). Moreover, within LNP, the biomass appeared to be highest for the mid-elevation

population, Lauribina Pass at 4200 m and, otherwise, it decreased with increasing elevation (**Figure 10b**). In ANCA, an average of 2136 dried rhizomes (range: 1964–2310) were needed to obtain one kilogram; while in LNP, an average of 1358 rhizomes (range:1001–1780) were required per kg. In both regions, the number of individuals needed to obtain one kilogram of dried rhizome generally increased with elevation.

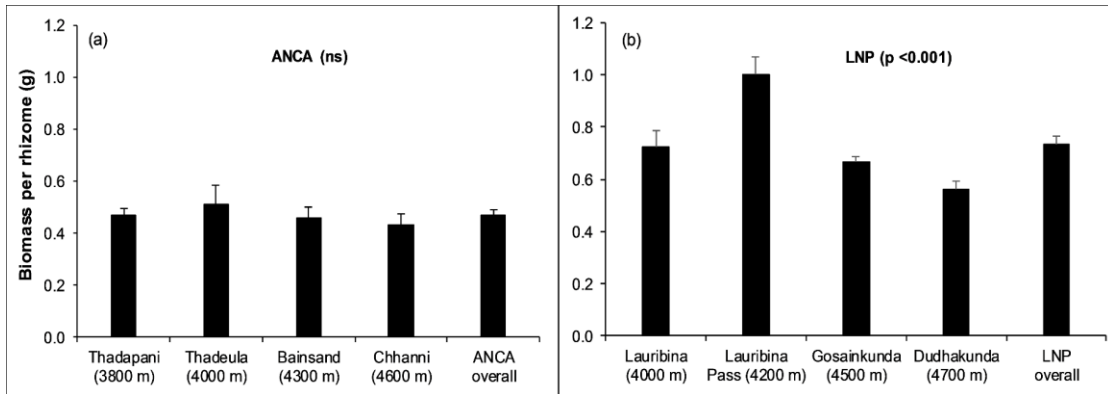


Figure 10: *Neopicrorhiza scrophulariiflora* population-wise rhizome biomass (Mean \pm SE) in Nepal's two regions: (a) Api-Nampa Conservation Area (ANCA) and (b) Langtang National Park (LNP). Kruskal-Wallis test was used to examine population variation (p -values stated in brackets)

3.4.4 Effect of Environmental Factors on Plant Density and Biomass

The environmental variables recorded in the sample plots varied within and between regions (**Table B.8**). Major differences between regions were observed for ground cover variables and disturbances. Shrub, rock, and moss/lichen cover were higher in LNP than in ANCA. Cover of herbs and graminoids were higher in ANCA than in LNP. The average scores of harvesting and grazing were remarkably higher in plots studied in ANCA than in LNP (**Table B.8**).

Out of twelve variables (including ‘population’) studied (**Table B.8**), only nine were used in GLMM after removing confounded variables. Moss/lichen cover was confounded with shrub cover, and bare ground cover was confounded with herb and graminoid cover. Shrub height revealed several missing values and so, it was excluded in the model. GLMM identified five variables that exhibited a clear association with plant density (**Table 4**). Rock cover, in general, did not show any notable effect on plant density when data from both regions were treated together. However, when data were treated separately for each region, rock cover was found to be associated positively with plant density in ANCA (Pearson correlation, $r = 0.323, 0.281, 0.320$ and 0.311 for

juvenile, adult vegetative, reproductive and total ramet density, respectively; $p < 0.010$; **Figure 11a**). By contrast, in LNP the corresponding relationship was negative ($r = -0.303$ and -0.275 for adult vegetative and total ramet density, respectively; $p < 0.010$; **Figure 11b**).

In the combined model, shrub cover also appeared to have a positive effect on ramet density (**Table 4**). When data were treated separately for each region, it emerged that increasing shrub cover was associated with increasing ramet density in LNP (**Figure 11d**) but not in ANCA. For individual stage classes in LNP, shrub cover showed a weaker positive relation with reproductive ramet density ($r = 0.269$, $p = 0.003$) than with juvenile ($r = 0.398$, $p < 0.001$) and adult vegetative ($r = 0.579$, $p < 0.001$) ramet density. By contrast, in ANCA, the shrub cover showed a negative relationship with ramet density (**Figure 11c**), but the relationship was only significant for reproductive ramet density ($r = -0.229$, $p = 0.012$).

Among all environmental variables studied, harvest disturbance had a negative effect on ramet density for all stage classes, whereas the effect of grazing was positive for juvenile and adult vegetative ramet densities (**Table 4**). A significant interaction between harvest impact and region further indicated that the negative effect of harvest on plant density was stronger in ANCA, and the effect of harvest was subtle for the LNP populations (**Table 5**). The weak positive effect of harvest on ramet density in LNP (positive interaction in **Table 5**) for all vegetative stages could be linked to the narrow range of harvest intensities.

The harvest impact showed different association patterns with rock and shrub cover in the two regions. In LNP, the harvest score exhibited no clear relation with shrub cover ($p > 0.050$), whereas the relationship was positive in ANCA ($r = 0.326$, $p < 0.001$). Contrary to this, in ANCA, the harvest impact score was negatively related with rock cover ($r = -0.268$, $p = 0.003$); whereas, in LNP, the relation was not significant. The positive relationship between harvest impact score and shrub cover further indicated that shrubland habitats in ANCA were preferred collecting sites for *Neopicrorhiza* harvesters. In general, while fitting variables in GLMM (**Table 5**), we detected a lower value of AIC when elevation (AIC = 2282.85) was included in models instead of populations (2287.90, against null model = 2385.51). This may indicate that part of the variability among the populations can be ascribed to differences in elevation (**Table 5**).

Table 4: Generalized linear mixed effect models reflecting effects of environmental conditions (Parameter estimate and SE: standard error) on ramet density (m^{-2}) of *Neopicrorhiza scrophulariiflora* at Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), in Nepal

Explanatory variable	Juvenile		Adult vegetative		Reproductive		Overall		
	Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	
ANCA	Intercept: Thadapani 3800 m	2.117***	0.180	3.695***	0.153	0.295	0.272	3.923***	0.157
	Thadeula 4000 m	-0.232	0.254	0.22	0.215	0.874*	0.342	0.205	0.22
	Bainsand 4300 m	-0.162	0.254	-0.206	0.213	0.382	0.364	-0.172	0.218
	Chhanni 4600 m	-0.213	0.255	-0.243	0.222	0.203	0.361	-0.258	0.227
LNP	Lauribina 4000 m	0.264	0.253	0.16	0.221	-0.24	0.367	0.202	0.226
	Lauribina Pass 4200 m	-0.259	0.263	-0.212	0.230	-0.676	0.369	-0.195	0.236
	Gosainkunda 4500 m	-0.169	0.271	-0.213	0.224	0.117	0.344	-0.189	0.231
	Dhudhakunda 4700 m	-0.346	0.274	-0.467*	0.229	-0.574	0.359	-0.414	0.235
Cover substrate	Shrub cover	0.230***	0.069	0.281***	0.069	0.294***	0.084	0.260***	0.071
	Graminoid cover	-0.237***	0.064			-0.368***	0.098		
Edaphic	Soil Ph	0.157*	0.072						
Disturbance	Harvest	-0.390***	0.072	-0.556***	0.072	-0.871***	0.088	-0.570***	0.074
	Grazing	0.335***	0.07	0.402***	0.071	-0.143	0.08	0.384***	0.072
	AIC: Full	1483.931		2210.60		865.266		2331.222	
	AIC: Null	1530.20		2269.50		973.90		2385.51	
	Observations (n)	240.00		240.00		240.00		240.00	

Plot was used as a random factor in the models that were calculated using a negative binomial distribution. The symbols $p < 0.0001$ '***', $p < 0.001$ '**', and $p < 0.05$ '*' represent different degrees of significance.

Table 5: Effect of environmental factors (Parameter estimate and SE: standard error) on *Neopicrorhiza scrophulariiflora*'s ramet density (m^{-2}), ramet biomass (g), and tissue density (g cm^{-3}) calculated by generalized liner mixed effect modeling in two regions of Nepal: Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP)

Explanatory variable		Juvenile		Adult vegetative		Reproductive		Total ramets		Rhizome biomass		Rhizome Tissue density		
		Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	Parameter estimate	SE	
Region	Intercept: ANCA	1.874***	0.126	3.441***	0.103	0.333*	0.161	3.684***	0.104	–	0.889*****	0.056	0.814***	0.093
	LNP	0.410*	0.173	0.454**	0.140	–0.076	0.226	4.126***	0.099	0.497***	0.084	0.215	0.139	
Harvest		–0.667***	0.094	–0.757***	0.100	–1.164***	0.129	–0.762***	0.101	–0.279***	0.053	–0.353***	0.087	
Elevation		–0.264	0.135	–0.360**	0.111	–0.435**	0.168	–0.354**	0.112	–0.118	0.060	0.251*	0.101	
Interaction	LNP*Harvest	0.805***	0.123	0.820***	0.131	0.296	0.177	0.796***	0.133	0.079	0.086	–0.044	0.144	
	LNP*Elevation	0.134	0.178	0.204	0.146	0.524*	0.218	0.211	0.148	–0.078	0.081	0.144	0.136	
AIC: Full		1481.752		2209.50		865.266		2326.979		234.20		657.40		
AIC: Null		1530.20		2269.50		973.90		2385.51		295.80		717.50		
Observations (n)		240.00		240.00		240.00		240.00		294.00		294.00		

Natural log transformation was performed to adjust for the skewed distribution of biomass and tissue density. Parameters were computed assuming a negative binomial distribution for density. For ramet density, 'Plot' was included as a random effect, while 'Subplot' was included as a random effect for biomass and tissue density of rhizomes. The symbols $p < 0.0001$ '***', $p < 0.001$ '**', and $p < 0.05$ '*' represent different degrees of significance.

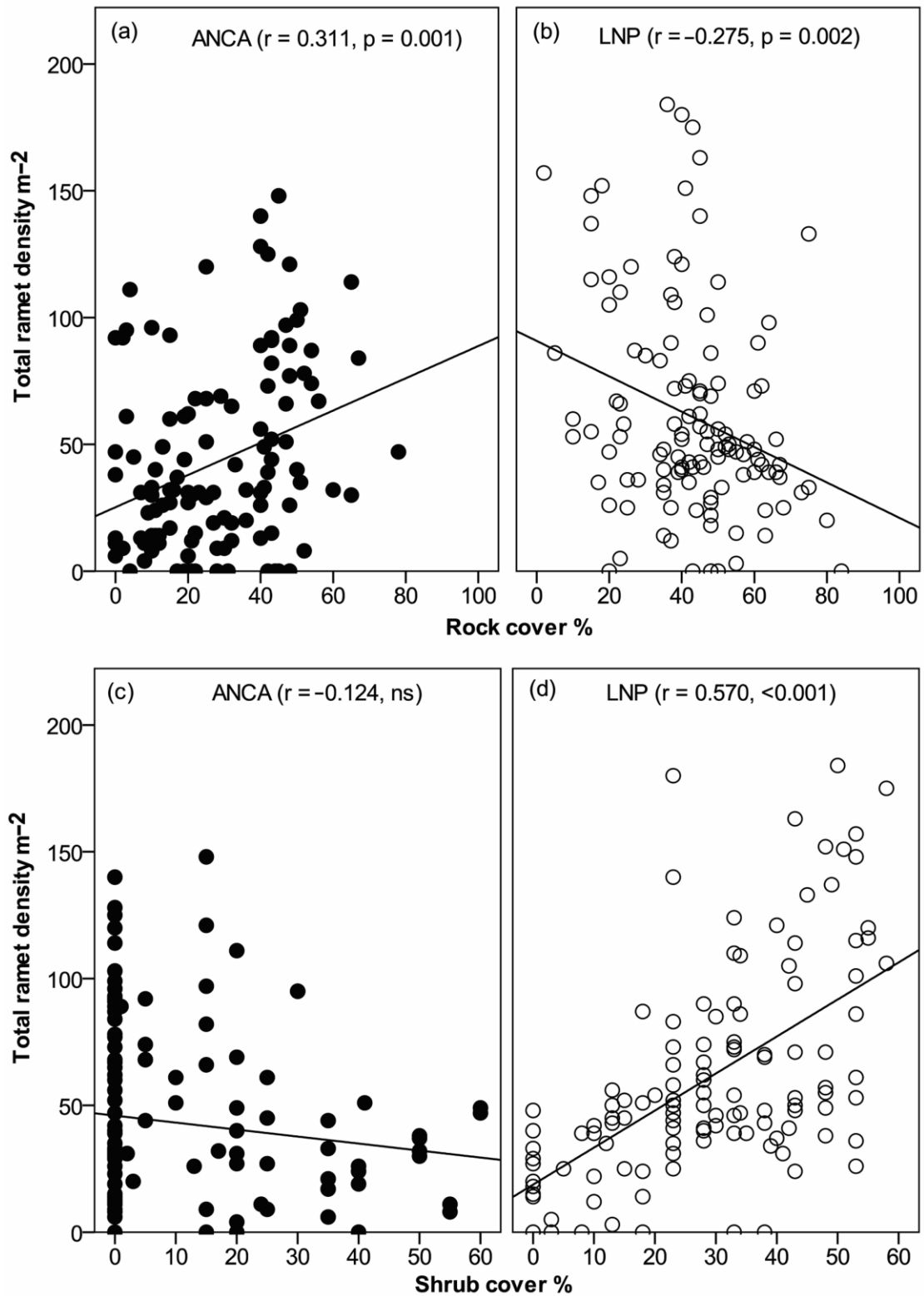


Figure 11: Relationship between *Neopicrorhiza scrophulariiflora* total ramet density (overall stages, m^{-2}) and (a) rock cover and (b) shrub cover in two regions of Nepal: Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP)

Elevation generally had negative parameter estimates, indicating that ramet density gradually decreases with increasing elevation. In contrast to the other stages, reproductive ramet density in LNP was weakly positively related to elevation (**Table 5**). Otherwise, the effect of elevation on ramet density was less negative in LNP than in ANCA, which could be related to the better protection characterizing LNP. Moreover, harvest was heavier at low elevations than further uphill ($r = -0.252$, $p = 0.006$, for ANCA and $r = -0.367$, $p = 0.001$ for LNP), presumably reducing ramet densities at low elevations.

Rhizome girth decreased with increasing elevation but this effect was more prominent in LNP ($r = -0.490$, $p < 0.001$) than in ANCA ($r = -0.162$, $p = 0.040$). Rhizome volume was not correlated with elevation in ANCA, but in LNP, it decreased with increasing elevation ($r = -0.591$, $p < 0.001$). Harvest negatively affected rhizome biomass in both regions and this effect was slightly lower in LNP than in ANCA (**Table 5**). We did not observe an effect of elevation on biomass (**Table 5**), but rhizome tissue density increased with elevation in both regions (**Table 5**). This indicates that tissue density was more sensitive to environmental variation along the elevation gradient than biomass. Additionally, this may suggest that older ramets with denser tissues were more prevalent at higher elevation, especially in populations in LNP (**Figure 12a,b**).

Ramet girth (cm) was higher in ANCA (mean \pm SE = 0.211 ± 0.008) than in LNP (0.176 ± 0.006 , $p < 0.001$). Likewise, mean rhizome volume (cm^3) was also higher in ANCA (0.36 ± 0.05) than in LNP (0.26 ± 0.02 , $p = 0.023$). On the other hand, the rhizome tissue density (g cm^{-3}) was considerably higher in LNP (5.53 ± 0.37) than in ANCA (2.66 ± 0.22 , $p < 0.001$). In addition, the mean rhizome biomass (dry weight in g) was about 1.5 times higher in LNP (0.74 ± 0.03) than in ANCA (0.47 ± 0.02 , $p < 0.001$). In ANCA, mean rhizome biomass did not deviate between the populations (**Figure 12a**). Moreover, within LNP, the biomass appeared to be highest for the mid-elevation population, Lauribina Pass at 4200 m and, otherwise, it decreased with increasing elevation (**Figure 12b**). In ANCA, an average of 2136 dried rhizomes (range: 1964–2310) were needed to obtain one kilogram; while in LNP, an average of 1358 rhizomes (range: 1001–1780) were required per kg. In both regions, the number of individuals needed to obtain one kilogram of dried rhizome generally increased with elevation.

the highly valued insectivorous fungus *Ophiocordyceps* has exceeded the total income from the harvest of all other MAPs (Negi *et al.*, 2014; Pouliot *et al.*, 2018). However, when valuable MAPs become scarce, collectors start collecting other highly valued products (Mulliken, 2000; Lange, 2004).

Since livelihood options in ANCA are constrained by its rugged terrain and remoteness, people supplement their income primarily by harvesting MAPs and other forest products (Pouliot *et al.*, 2018; Pyakurel *et al.*, 2018). In contrast, LNP is an attractive tourist destination in Nepal, in part because it features an important pilgrimage site (the sacred Gosainkunda lake), which is visited by tens of thousands of devotees every year (Shakya, 2016). People in LNP therefore supplement their income by engaging in tourism and hotel business instead of harvesting and trading forest and environmental products.

The broad geographic distribution of *Neopicrorhiza* in the Himalayas implies that this species is an easily accessible economic resource to many rural people (Olsen and Larsen, 2003; Olsen, 2005). The cash income earned by selling high-value medicinal plants, including *Neopicrorhiza* can be an appreciable supplement to local subsistence (Olsen, 2005). Conveniently, in ANCA, the traditional practice of MAP harvesting is legally permitted through the Management Council, formed based on Protected Area Management Regulation, which issues collection and transportation permits for all legal environmental products (HMG/N, 1978). All national parks in Nepal located at high elevation are managed under the Himalayan National Park Regulation (HMG/N, 1978) which also includes a provision to issue harvesting permits to selected MAPs within the park boundary. However, in LNP, permits to collect and trade MAPs have been provided only for *Ophiocordyceps* (personal communication with a conservation officer, 26 July 2018). It therefore stands to reason that, respondents in LNP might be less likely to report commercial collection of *Neopicrorhiza* than respondents in ANCA, where such collection is permitted. Nevertheless, some studies indicate that illegal harvest of MAPs could occur in both regions due to geographical remoteness and lack of regular invigilation mechanisms (Uprety *et al.*, 2011; Shrestha *et al.*, 2014; Pyakurel *et al.*, 2018).

3.5.2 Variation in Plant Density and Biomass

Our observations show that the variations in ramet density and rhizome biomass of *Neopicrorhiza* correlate with harvest intensities and physical habitat variables. The

effect of commercial harvesting appeared critical for plant density, rhizome size and biomass in ANCA. Results indicated that the plant density and biomass is 1.5 times higher in LNP than in ANCA, likely demonstrating that the former is better protected than the latter. Compared to LNP, in ANCA 1.6 times more ramets must be collected to obtain one kilogram (dry weight) of rhizomes. Based on our annual observations, a conservative estimate is that commercial collectors in ANCA collect approx. 364,000 rhizomes (average: 2136 rhizomes/kg) to achieve their harvest of 170 kg dry weight. This estimate suggests that commercial harvest may have a stronger effect on *Neopicrorhiza* populations than other medicinal rhizomatous Himalayan species like *Picrorhiza kurrooa*, where only 200–500 rhizomes are needed per kg of dry mass (Rai *et al.*, 2000; Uniyal *et al.*, 2011). Given the strong harvest pressure, we suggest an action plan to be prepared for ANCA to mitigate the risk of over-collecting *Neopicrorhiza* rhizomes in this region. A large variation in ramet density between study regions was observed for adult stages with lower density in ANCA than in LNP. Furthermore, a greater proportion of reproductive ramets in LNP than in ANCA may be an additional indication of the heavy collection pressure that affects *Neopicrorhiza* populations in ANCA. Moreover, Ghimire *et al.* (2005; 2008) stated that alpine MAPs, including *Neopicrorhiza*, should be left unharvested for at least 5–10 years to allow sufficient time for individuals to grow and reproduce between the harvests.

Plant density and biomass decreased with increasing elevation, indicating how environmental stresses (e.g., lower temperature, shorter growing time) constrain growth at high elevations (He *et al.*, 2017). However, in LNP reproductive ramet density increased weakly with elevation (**Table 5**) but, otherwise, the overall effect of elevation was weakly negative in LNP and clearly negative in ANCA. The increase in reproductive ramet density in LNP along the elevation could reflect a reduced harvesting pressure with increasing elevation in combination with a tendency of plants to be more likely to grow to reproductive maturity when growing in less disturbed conditions. However, in ANCA, the negative effect of human disturbance on *Neopicrorhiza* was evident, also for the reproductive stage and regardless of elevation. This finding is consistent with Rusterholz *et al.* (2009), who observed that sexual reproduction in the rhizomatous clonal herb *Anemone nemorosa* increased with declining human disturbance.

3.5.3 Major Habitat Determinants

In LNP, the shrub cover and moss/lichen cover showed a positive relationship with *Neopicrorhiza* density ($r = 0.787$, $p < 0.0001$). Hence, it could be suggested that shrub vegetation with substantial moss/lichen ground cover maintains a high moisture level that favors *Neopicrorhiza* populations and leads to increased density. Positive effects of shrub facilitation, such as reduced evapotranspiration, improved thermoregulation, and soil amendment by litter accumulation and decomposition have been reported in previous studies (e.g., Ballantyne and Pickering, 2015). However, in LNP the shrub cover generally decreased with increasing elevation. Based on the density and biomass estimates in LNP, we suggest low elevation populations (<4500 m) are likely more resilient to low-intensity harvest, in part due to facilitative effects offered by shrub cover.

However, in ANCA, rock cover showed a positive relationship with plant density (**Figure 10a**), whereas the relationship between shrub cover and plant density was not significant. Our interviews with MAP collectors revealed that their impression was the opposite, as they believed that, compared to other habitats, shrubland habitats had higher biomass of *Neopicrorhiza* per unit area, which allowed them to collect larger amounts within a given period of time (data not shown). Commercial collectors usually harvest intensively in order to maximize yield while minimizing effort and time. Hence, they prefer to harvest in dense populations where high quantities of rhizomes are available (Ghimire *et al.*, 2004). We therefore suggest that the observed higher density of *Neopicrorhiza* in rocky habitats in ANCA may be due to the preference of harvesters for the shrubland habitats at lower elevations.

In our study, grazing showed a positive effect on ramet density except for the reproductive stage (**Table 4**). Our field observations indicated that *Neopicrorhiza* is unpalatable to herbivores, which could be linked to secondary metabolites, such as terpenoids, cucurbitacin, kutkisterol, and steroids present in the plant (Li *et al.*, 2014). Additionally, in alpine meadows, grazing tends to decrease competition for light without altering functional diversity of foliar traits (Niu *et al.*, 2016). Hence, the removal of species other than *Neopicrorhiza* by grazing reduces competition for light and provides the species with suitable conditions for vegetative regeneration. Indeed, the guerrilla growth form of *Neopicrorhiza* allows it to multiply as soon as it finds open space (Humphrey and Pyke, 1998; Ghimire *et al.*, 2005).

Clonal growth is a successful strategy for persistence of plant populations in stressful environment like those of the Himalayas (Dvorský *et al.*, 2013; 2015; Herben *et al.*, 2014). Based on the parameter estimates obtained for juvenile and adult vegetative ramet densities it appears that for given levels of harvest and elevation, densities were higher in LNP than ANCA (**Table 5**). Ghimire *et al.* (2005) also found higher values of *Neopicrorhiza* density at low levels of harvesting. The plagiotropic growth of the plant enhances its ability to adapt to extreme environmental conditions where the competition from orthotropic plants is less important (Ghimire *et al.*, 2005; Katayama *et al.*, 2015). The ramets are only partly autonomous, which enables them to benefit from nutrients (nitrogen, phosphorus, potash, and urea) and photosynthates (sugars, amino acids, and hormones) made available by already existing ramets of the same genet (Lei, 2010). Previous studies (Evette *et al.*, 2009; Lei, 2010; Johansen *et al.*, 2016) found that the budding potential and ramet production of clonal herbs in disturbed environments is higher than in undisturbed environments. Consequently, the clonal growth and fugitive establishment of *Neopicrorhiza* could partly remediate the impact of low-intensity harvest in disturbed populations.

3.6 Conclusion

The present study provides baseline evidence on plant utilization systems, population differentiation, and habitat conditions of *Neopicrorhiza* across a conservation gradient. Both plant density and biomass indicate that a low harvest intensity had no adverse effect on the populations, although quantification of the optimum level of harvest remains to be done and is therefore an aim of ongoing research. Market-driven harvesting and pre-mature exploitation are the most important issues for long-term management. The high number of harvesters in the open-access region appeared to have a strong negative impact on *Neopicrorhiza* populations, whereas in the protected region such clear effects were not observed. Shrubland habitats exhibited high plant densities in the protected region (LNP), but in the open-access region (ANCA), heavy exploitation seemed to occur in such habitats and so, a higher plant density occurred in rocky areas instead. To ensure that the number of reproductive individuals left in each harvested population is sufficient to maintain population viability, we propose regulating harvest such as by introducing rotational harvest systems and *in situ* management of *Neopicrorhiza* populations. Additionally, we suggest regulating management through implementing community monitoring of harvest, involving local

users, collectors, and park officials. Finally, we recommend analyzing the effects of harvest on populations dynamics to estimate suitable limits of annual harvest and develop strategies for sustainable management.

CHAPTER 4

4. POPULATION RECOVERY AFTER IN SITU EXPERIMENTAL HARVEST¹

4.1 Abstract

Medicinal and aromatic plants (MAPs) are essential for maintaining human health and generating immediate cash income for rural livelihoods, but trade-mediated overexploitation and habitat destruction impeded the sustainable management of natural habitats. Management plans for many of the wild-extracted MAPs lack any empirical data on post-harvest recovery of density, or of key demographics such as fruit-setting and seed formation. We hypothesized that harvest affects negatively the plants' vital functions, survival, and long-term viability, but that not all levels of harvests would be detrimental to density, growth, and reproduction, and that the restoration capacity would allow some level of sustainable extraction. To test this hypothesis, we applied simulated harvest field experiments to a commercially threatened MAP, *Neopicrorhiza scrophulariiflora*, in eight populations along an elevation gradient (3800–4700 m), including four in a restricted-access and four in an open-access site in the Nepal Himalaya. We found that density and reproductive outputs varied significantly among the harvest treatments and covaried with the pre-harvest condition. At low-elevation plots in the restricted-access sites, both density and reproductive output recovered within three years after experimental harvest from up to 50% removal, and in a single year from 25%. However, in the open-access site, recovery to the pre-harvest level was achieved only at 25% treatment after a one-year's interval. Harvest recovery was slower at higher elevations (>4250 m), and plots harvested more intensively (>50% extraction) recovered very slowly. Our results show the importance of using spatially and temporally specific harvesting strategies to manage populations sustainably.

Keywords: Alpine plants; over-exploitation; vital traits; post-harvest monitoring; repeated measures; sustainability

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4.2 Introduction

The collection of non-timber forest products for food, medicine, and religious or cultural use is a common practice by people worldwide and has roots stretching back to human pre-history (Ticktin, 2015; Hardy, 2018). Among these products, the harvest of wild medicinal and aromatic plants (MAPs) remains significant to rural people both for health and for income through trade. Demand for wild-harvested MAPs is expected to increase due to consumer preference, perceived medicinal efficacy, and constraints on cultivation (Fennel *et al.*, 2004; Chen *et al.*, 2016). Achieving a sustainable harvest of MAPs, particularly in developing regions of the world, is a challenge and dependent on ecological, social, economic, and historical settings (Gaoue *et al.*, 2014; Ticktin, 2015). However, this is seen as a promising way to support both biodiversity and livelihoods, with commercialization to create employment opportunities, overcome poverty, and reduce income disparities in cash-poor rural communities (Olsen, 2005; Jensen and Meilby, 2008; Ticktin, 2015). To this end, developing strategies for sustainable harvest is not only a means for plant conservation but also essential for the welfare of many rural peoples (Olsen, 2005; Gaoue and Ticktin, 2007).

Scientific assessments of wild MAPs have established a variety of plant-specific, empirically-supported approaches for sustainable harvest (Ticktin 2015; Schmidt *et al.*, 2019). For instance, density, recruitment, and survival of clonal perennial herbs are positively correlated to low-intensity and rotational harvesting (Sanders and McGraw, 2005; Ghimire *et al.*, 2005; 2008). Management regimes and harvest methods, including traditional harvest methods, can also positively affect growth and reproduction (Freckleton *et al.*, 2003; Gaoue *et al.*, 2011; Poudeyal *et al.*, 2019). Harvest methods may also promote an increased amount of plant material that can be extracted sustainably, relative to a control (Gaoue *et al.*, 2011; 2014) but the magnitude of this impact is determined by the growth forms, regenerating capacity, density of population, and the nature and intensity of harvesting (Ticktin and Nantel, 2004; Ndangalasi *et al.*, 2007; Schmidt and Ticktin, 2012).

Given the possibility of science to potentially inform sustainable harvest of MAPs, it is surprising that there have been very few studies on sustainable harvesting strategies from the Nepal Himalaya (Ghimire *et al.*, 2005; 2008; Rokaya *et al.*, 2017), despite the importance of MAPs in the area. Among the estimated 2000–2500 MAP species from the Nepal Himalayas, 300 are reported as traded (Pyakurel *et al.*, 2019).

Many of these are highly vulnerable, particularly the 30 high-demand species, which are restricted to elevations above 2000 m (Pyakurel *et al.*, 2019). Slow growth and limited seed set, recruitment, and expansion make high-altitude species especially vulnerable to exploitation (Rana *et al.*, 2020). Due to their geographic isolation and location at the edge of climate gradients, high-elevation MAPs are thought to be more susceptible to the effects of climate change than traded low-elevation MAPs (Poudeyal and Ghimire, 2011; Manish and Pandit, 2019; Rana *et al.*, 2020).

To identify factors determining ecologically sustainable use and effective management strategies in the sensitive Himalayan ecosystem, we studied synergistic demographic response to harvest intensities in *N. scrophulariiflora*, a threatened and increasingly traded clonal herb distributed at 3000–5000 m in the eastern and central Himalayas. Our objectives were to examine the effects of *in situ* harvesting on plant density and fitness-related (reproductive) traits through an annual census across the environmental gradient. We hypothesized that harvest affects negatively the plants' vital functions (sexual reproduction), survival, and long-term viability, but that not all levels of harvests would be detrimental to density, growth, and reproduction, and that the restoration (regeneration) capacity determined by the respective environment would allow some level of sustainable extraction. We asked: 1) How does density vary with extraction intensity and other factors (time since first harvest and elevation)? 2) Does the response to different harvest intensities vary along environmental gradients? 3) In what ways are reproductive outputs (fruit formation, seed sets, and fecundity) linked with harvesting and environmental gradients? (4) What are sustainable harvest levels for populations in different environmental conditions? What management strategies may foster sustainable conservation?

4.3 Material and Methods

4.3.1 Study Area

The Api-Nampa Conservation Area (ANCA: 29.97°N and 80.96°E) in the north-west and Langtang National Park (LNP: 28.09°N and 85.42°E) in the north-central Nepal were both protected regions where field surveys and monitoring were done (**Figure 13**). Traditional subsistence agriculture, livestock farming, and natural resource utilization are the main livelihoods in both sites, but while the trade of MAPs is a mainstay for the rural economy in ANCA, tourism, and hotel business are very important in LNP. Geographical fragility and steepness, inaccessibility and poor infrastructural

development, and limited agricultural productivity are major limitations to development, leading to poverty for up to 50% of the people in LNP and 70% in ANCA (Pyakurel *et al.*, 2018; Poudeyal *et al.*, 2019). ANCA, established in 2010, is located in Darchula District and is regarded as open for regulated trade of environmental products with prior permission from the Government of Nepal. Thus we describe it as a de facto ‘open-access’ area for commercial harvest. LNP was established in 1976 extending over parts of Rasuwa (57% of its area), Sindhupalchok (36%), and Nuwakot (3%) districts. Commercial collection of MAPs in LNP has been restricted except for a limited opportunity to collect *Ophiocordyceps sinensis* (caterpillar-fungus) for trade, and we, therefore, describe it as a ‘restricted-access’ area for commercial harvest of *Neopicrorhiza*. Due to the remoteness of the locations, the government's lax enforcement, and the great poverty, unlawful gathering of MAPs may occur in both locations regardless of the regulation variations (Poudeyal *et al.*, 2021).

Pyakurel *et al.* (2018) stated that 19 tons of *Neopicrorhiza* were sold from Darchula in the fiscal year 2014/015. No sales were recorded from the restricted-access areas of LNP (Poudeyal *et al.*, 2019), but from the non-protected area in the Rasuwa, 21,750 kg was sold in 2016/017 (unpublished data: District Division Forest office, Rasuwa). The market demand for MAPs is considered to be unstable in Nepal and generally, extraction and trade of the species depend on collection permits issued by the local government and interest among the traders (Olsen, 2005; Pyakurel *et al.*, 2018). The climate is cool and dry in winter but wet and moist in summer at both sites. Winter precipitation occurs as snow beginning in November, and melting occurs from the end of April in LNP and May in ANCA (see: Poudeyal *et al.*, 2019 for detailed description).

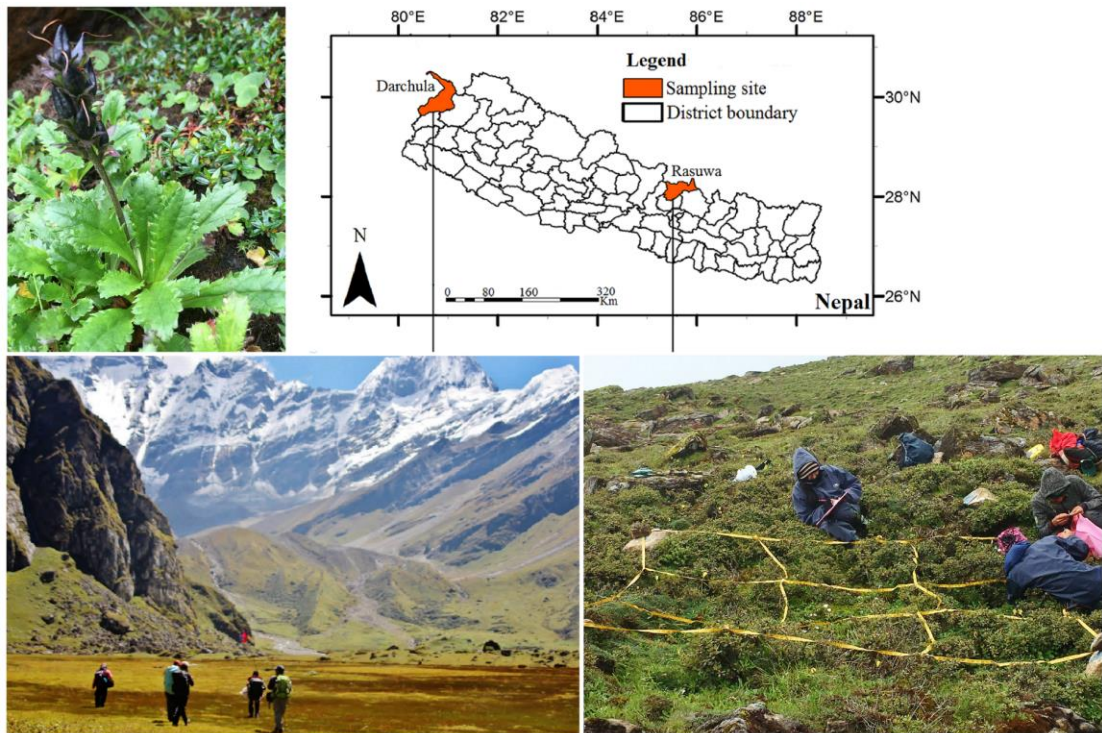


Figure 13: Details of the study species and area: a mature individual of *Neopicrorhiza scrophulariiflora* (top-left, size varies from 5 to 25 cm tall); sampling sites (top-right); landscape with team members at the background in Api-Nampa Conservation Area, at Darchula (bottom-left); and close-habitat with sampling-monitoring of the permanent plot in Langtang National Park (bottom-right), at Rasuwa district in the Himalaya Nepal.

4.3.2 Study Species

Neopicrorhiza scrophulariiflora (hereafter *Neopicrorhiza*, Family: Plantaginaceae, Synonym: *Picrorhiza scrophulariiflora* Pennell, Commercial name: Kutki) is a clonal herb (**Figure 13**) growing at elevations of 3500–5000 m, and distributed in Nepal, China, Sikkim, and Bhutan. The plant bears dark-blue flowers, generally, in May, fruiting follows in July, and the seed is wind-dispersed in October. On average, an individual ramet bears 5–9 ovoid fruits, each with 30–50 seeds. No newly emerged ramets produced flowers within the duration of our study, showing that each ramet needs more than five years for sexual reproduction. The plant has no persistent soil seed bank and seedling recruitment is very limited (Ghimire *et al.*, 2005). However, regeneration generally occurs from old ramets which bear extensive networks of rhizomes with lateral roots and exhibit a ‘guerrilla growth’ strategy that enables the species to enhance potential resource capture from resource-rich microhabitats (Ghimire *et al.*, 2005).

The wild harvest of the underground rhizome for trade has a century-long history (Ghimire *et al.*, 2005; Olsen, 2005). The plant is regarded for its cathartic and purgative qualities as well as its ability to reduce fever, high blood pressure, conjunctivitis, bile reflux, and digestive aches (Ghimire *et al.*, 2005; Poudeyal *et al.*, 2019). The high content of bioactive phytochemicals, such as iridoid glycosides and kulkosides compared to closely related *Picrorhiza kurrooa* Benth. (western Himalayan CITES-listed species) has increased the demand for *N. scrophulariiflora* by pharmaceutical industries (Kumar *et al.*, 2016).

In our study sites, the *Neopicrorhiza* rhizomes are harvested in May–June (premature) for domestic consumption, and July–October for trade. The sales prices of air-dried rhizomes range from 600 Nepalese rupees (Nrs.) per kg (Nrs. 100 \approx 1 USD, at the time of data collection) at the village of collection to 1000 Nrs. per kg at the Nepal-India border. In Nepal, *Neopicrorhiza* is legally protected, and collection, trade, and export without permission are banned, but a large quantity is still extracted illegally (Pyakurel *et al.*, 2018). Olsen (2005) reported that 175–770 tons of rhizomes are annually exported from Nepal, which accounts for 66% of the global market. The plant has been listed as critically endangered in China (Wangchuk and Olsen, 2010), vulnerable to endangered in Nepal (Bhattarai *et al.*, 2002), India (Kumar *et al.*, 2016), and Bhutan (Dong *et al.*, 2007), according to a number of studies, but the IUCN has not yet placed the species on its red list of threatened species.

4.3.3 Sampling Procedure

Field observations in 2014 determined four populations in ANCA and four in LNP at elevations between 3800 and 4700 m (the elevational range of *Neopicrorhiza* at these sites). The populations were located at Thadapani (3800 m), Thadeula (4000 m), Bainsand (4300 m), and Chhanni (4600 m) in the upper Chamelia Valley within ANCA; Lauribina (4000 m), Lauribina Pass (4200 m), Gosainkunda (4500 m) and Dudhakunda (4700 m) in LNP within the catchment area of the Trishuli River. Local people had easy access to all populations for the collection of MAPs. The lowest elevation (<4000 m in ANCA and <4200 m in LNP) populations were more frequently harvested than populations at higher elevations. Shrub cover was higher at lower elevations; rock cover and grass cover were relatively higher towards higher elevations at both sites (Poudeyal *et al.*, 2019).

Following Ghimire *et al.*'s (2005) methods for sampling in each population, we established three plots (3m × 3m) subjectively at 20–30 m of inter-plot vertical distance in 2015. To locate permanent plots, we further considered secluded locations with uniform distribution and high density of plants, and no indications of recent harvest or other anthropogenic activities. Each permanent plot was divided into nine 1 m² subplots, of which four were chosen for thorough observations of ramet density at the corners and one at the middle (**Figure 13**). In Dudhakunda (4700 m) at LNP, the distribution of *Neopicrorhiza* was quite clumpy, we only recorded ramets in four sampled subplots from two of the three plots and that unrecorded subplots were excluded in subsequent analysis. In total, we sampled 24 plots at the north-facing slope in each site, with 120 and 118 subplots in ANCA and LNP, respectively, for final analysis.

4.3.4 Vital Functional Traits: Reproductive Output and Seed Attributes

Along with ramet density, we recorded the number of fruiting bodies (but not individual seeds) for each reproductive ramet in each subplot prior to harvest treatment in 2015 and monitored the development annually until 2018. Seeds were not collected in experimental subplots so as to allow the natural processes to take place and, therefore, we randomly selected 30 healthy ramets (from different source plants at least 5 m away) around the permanent plots in each population. Of each ramet, three healthy and fully developed fruits at the bottom, middle, and top of the inflorescence were chosen. Seeds from each fruit were extracted separately. To calculate seed output per ramet, the average value was multiplied by the fruit number of each ramet. The same measurement was done repeatedly in the following years: 2016 and 2017 for ANCA, and until 2018 for LNP populations.

We conducted *in situ* seed germination experiments in each population. In 2015, we collected triplicate samples in each population consisting of five fully ripened fruits and extracted the seeds from these. The air-dried seed samples were stored in muslin mesh bags at the location of each population. In July 2016, at each population, triplicate nursery seed beds of 1 m² were established in the vicinity of a density plot where the mother plants were completely absent. A homogenous mixture of morphologically intact seeds (seed number per fruit (mean ± SE) = 40.32 ± 2.09) from five fruits was sown. Altogether, we employed 200 seeds per population and 800 seeds per study site for the field-germination assessment. The average recruitment percentage

(0.375% in ANCA and 0.625% in LNP) was multiplied with the seed number of each flowering ramet to calculate the potential fecundity (seed number \times proportion of seedling established from seed) in each subplot.

4.3.5 Harvest Treatments

After morphological and reproductive recording, we applied harvest treatments on ramet density in each subplot for all the populations and monitored the development from 2015 to 2017 (for ANCA) or 2018 (for LNP). In the fruiting season of 2015, we received prior verbal consent and asked herbal practitioners (trade-related harvester in ANCA and local healer in LNP) to apply treatments in a way that simulates actual harvest practice. We randomly assigned each of the five subplots to one of five treatment levels: removal of 0% (control), 25%, 50%, 75%, and 100% of the ramets. Although the harvesting practice was to uproot the best-grown ramets, for these treatments we asked them to select from all developmental stages regardless of size and ease of access, and harvest homogeneously across the treated subplots. In the case of 100% harvest, we attempted to harvest all aerial and underground parts, but due to the guerilla growth strategy and root fragments remaining after harvest, we did observe recruitment of ramets in subplots with 100% removal (as in Kindscher *et al.*, 2019).

In each subplot, all individuals remaining after harvest were marked with numbered aluminum tags fixed in the ground near each ramet. Subplot boundaries were marked with a painted stone, and we requested local harvesters, livestock herders, and hoteliers not to pick the plants from within these subplots. Plots were annually censused from July/August up to 2017 in ANCA and 2018 in LNP to monitor changes in population survival, structure, regeneration, mortality, and growth status (flowering and fruiting).

4.3.6 Data Analysis

We used Kindscher *et al.*'s (2019) method for the data analysis. Except for between-sites and within-site treatments at pre-harvest conditions, we analyzed and interpreted the data from ANCA and LNP independently because the experiments had different temporal ranges. To ascertain if harvest treatments had an impact on all response variables at each site, we created a mixed-effect model for repeated measures ANCOVA in R (Version: 3.6.2; R Core Team, 2019) using the *lme* function of the *nlme* package. The response variables were ramet density, mortality, and average annual values of the vital functional traits: reproductive investment (totaling all reproductive structures: aborted bud, flower,

and intact/aborted fruit), fruit abortion percentage, intact fruit set, seed production, and seed fecundity per ramet. Plots within the population were included as a random factor and were also represented by a first-order autocorrelation structure to control for autocorrelation among the repeated measurements. Other variables were considered fixed factors. Altogether, this led to 14 models, seven for each study site.

The density was the target variable for the harvest treatment, and for 100% harvested subplots no flowering individuals were recorded within the duration of our study. So, reproductive traits become irrelevant for these subplots, and hence, records for this treatment were eliminated from the datasets used in analyzing the effect of harvest treatment on the vital functional traits. Similarly, we entered a missing value of mortality for 100% treated subplots in the 2016 records. At both sites, two lower elevation populations were categorized as ‘lower alpine (<4250 m)’, and two populations were categorized as ‘upper alpine (>4250 m)’. We tested for the effects of harvest treatment, year, and elevation. To find differences between the harvest treatment levels, we ran Tukey's post-hoc multiple comparison tests using the *glht* function in the *multcomp* package. The pre-harvest density and reproductive traits in each subplot, measured just before the harvest treatment (2015), showed significant ($p < 0.05$) variation among the populations in each study site, so they were treated as a covariate in each model (as in Kindscher *et al.* 2019). We used square-root transformation for density, log-transformation ($\text{Ln}+1$) for other count data, and percentage data were logit transformed to improve the normality of the residuals.

4.4 Results

4.4.1 Ramet Density Restoration

The ramet density monitoring between 2015 and 2018 provided strong evidence for harvest recovery differentiation across the sites, among all populations, over time, and the elevation at different levels (**Tables 6 and 7, Figure 14, Tables B.9–B.11**). Although, the average pre-harvest density (m^{-2}) did not vary when treated between ANCA (mean \pm SE: 87.55 ± 7.82) and LNP (85.96 ± 6.97 , $p > 0.05$). In the meantime, it also did not vary when treated individually between lower and upper alpine populations ($p > 0.05$) in both sites. However, ramet density recovery varied significantly with harvest treatments and with pre-harvest density in both sites (**Table 6, Table 7**). The elevation effect was more evident in those recoveries in ANCA populations than in LNP. In general, we observed a more rapid increase in density in LNP than in ANCA for all harvest intensities

(including control), and the effect of time ($p < 0.01$) and the interaction between time and harvest were strong at both sites ($p < 0.001$). Upper alpine populations in ANCA showed a slower increase in density compared to lower alpine populations ($p < 0.001$), whereas elevation alone did not show a significant effect in LNP ($p > 0.05$). Furthermore, a significant interaction between harvest intensity and elevation at both sites showed that the negative effect of harvest increased with increasing elevation ($p < 0.001$, $p < 0.05$ for ANCA and LNP, respectively).

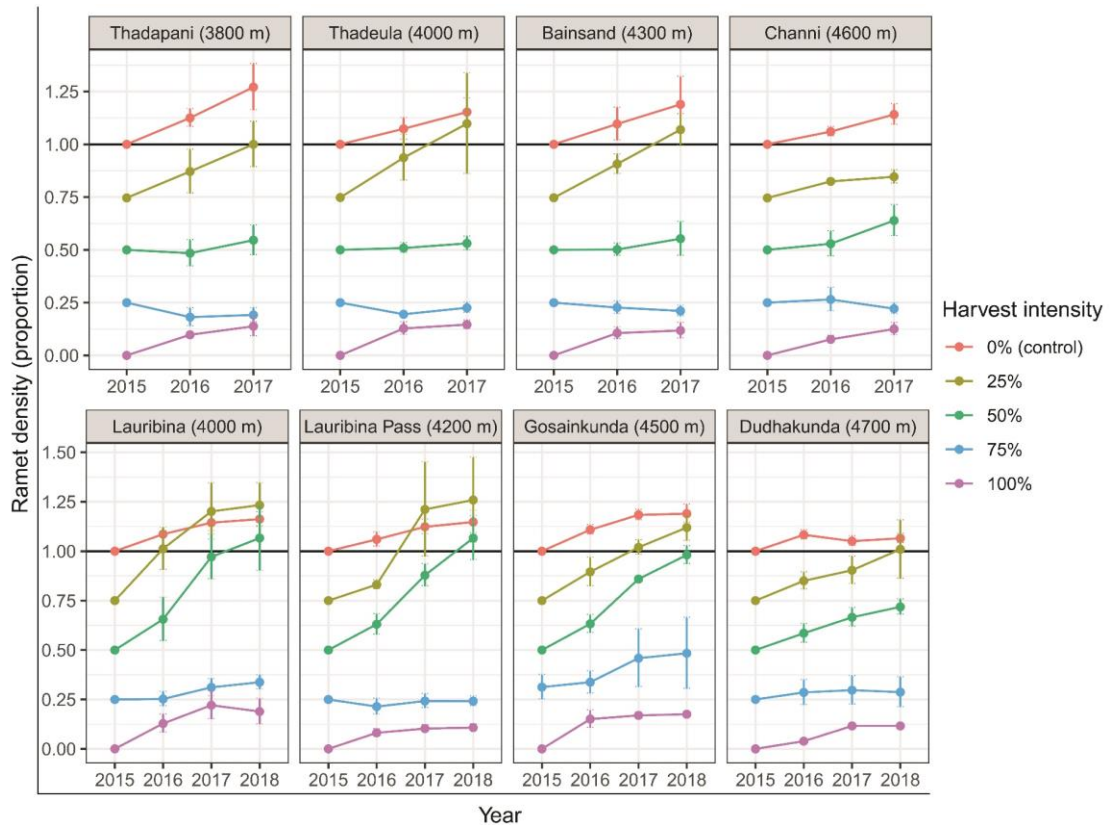


Figure 14: Ratio (mean \pm SE of proportion) of pretreated ramet density (m⁻²) of *Neopicrohiza scrophulariiflora* prior to harvest treatment made in 2015 to a relative measure (cumulative restoration) of annual postharvest ramet density along with populations in Api-Nampa Conservation Area (top panel, annually censused up to 2017), and Langtang National Park (bottom panel, annually censused up to 2018), in Himalaya Nepal

In ANCA, except at Channi (4600 m), subplots with 25% harvest had recovered 100% of their pre-harvest ramet density by 2017, during which time density in the control subplots had increased by 18% (**Figure 14**). By contrast, the densities of subplots subjected to greater harvest intensity remained low (43%, 79%, and 87% less dense than the pre-harvest levels for subplots with 50%, 75%, and 100% harvest, respectively). In lower alpine populations, 21%, and 5% increases in ramet density were observed for control and 25% treatment subplots but, as of 2017, increases in ramet

density of 46%, 79%, and 86% ramets were still needed to reach pre-harvest densities for the heavier harvest treatments (**Table 6**). In the upper alpine populations, a 16% increase in the density of ramets was observed only in control subplots by 2017, but in all other cases, the density remained much lower than the pre-harvest level (**Table 7, Figure 14**).

Table 6: Mean (\pm SE) pre-harvest ramet density m^{-2} prior to harvest treatment made in 2015 and its relative measure of cumulative change (annual restoration %) of *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area and Langtang National Park at different elevations [LA: Lower Alpine (<4250 m) and UA: Upper Alpine (>4250 m)] in Himalaya Nepal

Variable	Year	Elevation level	Harvest treatment level				
			0% (control)	25%	50%	75%	100%
(a) Api-Nampa Conservation Area							
Pre-harvest density	2015	LA	68.00 \pm 10.16	56.44 \pm 12.57	105.00 \pm 19.53	176.67 \pm 37.03	99.83 \pm 19.59
		UA	54.83 \pm 9.91	36.67 \pm 6.24	64.00 \pm 17.32	102.67 \pm 30.12	110.17 \pm 18.70
Restoration %	2016	LA	9.95 \pm 3.19	-9.26 \pm 6.85	-50.36 \pm 3.04	-81.16 \pm 1.92	-88.76 \pm 1.59
		UA	7.85 \pm 3.69	-13.03 \pm 2.97	-48.46 \pm 3.01	-75.41 \pm 2.96	-90.92 \pm 1.62
	2017	LA	21.20 \pm 6.27	5.25 \pm 11.82	-46.17 \pm 3.49	-79.15 \pm 2.00	-85.79 \pm 2.27
		UA	16.56 \pm 6.33	-3.73 \pm 6.21	-40.41 \pm 5.23	-78.41 \pm 1.65	-87.86 \pm 2.13
(b) Langtang National Park							
Pre-harvest density	2015	LA	91.00 \pm 10.83	51.78 \pm 10.40	55.67 \pm 13.42	90.67 \pm 22.92	100.33 \pm 21.85
		UA	84.67 \pm 15.37	40.22 \pm 8.95	67.33 \pm 18.15	162.67 \pm 21.23	118.25 \pm 32.96
Restoration %	2016	LA	7.29 \pm 1.85	-7.87 \pm 6.37	-35.71 \pm 5.45	-76.69 \pm 2.57	-89.52 \pm 2.50
		UA	9.56 \pm 1.62	-12.72 \pm 3.92	-41.34 \pm 3.14	-71.56 \pm 3.00	-87.72 \pm 4.22
	2017	LA	13.37 \pm 2.56	20.61 \pm 12.41	-7.53 \pm 5.98	-72.35 \pm 2.95	-83.80 \pm 4.20
		UA	11.71 \pm 3.40	-3.87 \pm 4.36	-23.23 \pm 5.06	-69.42 \pm 3.52	-84.40 \pm 1.69
	2018	LA	15.51 \pm 2.99	24.58 \pm 10.81	6.64 \pm 8.91	-71.07 \pm 2.89	-85.15 \pm 3.48
		UA	12.75 \pm 3.63	6.44 \pm 7.68	-14.36 \pm 6.55	-70.44 \pm 3.56	-83.96 \pm 1.61

The plant populations were censused every year between 2015 and 2017 for former and until 2018 for later site.

Across harvest intensity levels, the Tukey post-hoc test identified only recovery differences ($p < 0.01$) between the control (0%) and higher harvest levels (50%, 75%, and 100%), and between 25% and the highest levels (75% and 100%) (**Table 7**). Similarly, in LNP, subplots with 25% harvest had recovered and exceeded pre-harvest density by 8% in 2017 (increasing further to 16% by 2018), during which time control subplots increased by about 14%.

Table 7: Repeated-measures ANCOVA of *Neopicrorhiza scrophulariiflora* for testing the effects of initial density (D0), harvest treatment (H), time (years: Y) and elevational level (EL) on ramet density (m-2) and reproductive activities per ramet in (a) Api-Nampa Conservation Area (ANCA), north-western Nepal, where populations were censused annually between 2015 and 2017; and Langtang National Park (LNP), north-central Nepal, where populations were censused annually between 2015 and 2018

Variables		Density	Mortality	Reproductive investment	Fruit abortion %	Intact fruit number	Seed production	Seed fecundity
		(a) Api-Nampa Conservation Area (ANCA), north-western Nepal						
Between-subject effects	Density (D0)	120.28***	52.17***	3.01	1.30	5.45*	4.90*	14.66***
	Harvest treatment (H)	396.96**	9.42***	50.84***	23.12***	51.44***	54.41***	45.50***
	Elevation level (EL)	17.78***	0.22	8.09**	3.48	15.13***	6.46*	15.95***
	H×EL	6.73***	1.28	4.70**	2.88*	6.74***	5.71**	9.49***
	D0×H	35.56***	1.29	1.90	0.81	0.91	1.33	0.92
H*Tukey post-hoc contrast	0–25%			-2.92*				
	0–50%	-3.409**		-3.00*				
	0–75%	-4.843***						
	0–100%	-5.893***	2.89*					
	25–75%	-3.565**						
	25–100%	4.70***						
Within-subject effects	Year (Y)	4.06*	2.49	0.00	0.15	15.32***	11.19**	16.60***
	Y×D0	26.33***	33.23***	1.31	1.25	6.84*	5.71*	18.79***
	Y×EL	18.30***	0.33	7.43**	0.28	30.08***	18.16***	30.30***
	Y×H	81.79***	6.41***	20.80***	11.71***	27.13***	28.25***	24.45***
	Y*EL*H	1.26	2.47*	2.04	1.81	4.94***	4.15**	6.71***
(b) Langtang National Park (LNP), north-central Nepal								
Between-subject effects	Density (D0)	172.09***	51.33***	20.59***	0.36	30.13***	28.85***	50.59***
	Harvest treatment (H)	374.58***	3.36*	44.75***	25.35***	41.28***	44.78***	37.90***
	Elevation level (EL)	2.93	7.92**	9.23**	0.53	7.26**	2.44	7.83**
	H×EL	3.42*	2.07	3.95**	4.27**	3.63*	3.10*	4.61**
	D0×H	54.51***	6.93***	2.28	2.97*	1.25	1.02	1.07
H*Tukey post-hoc contrast	0–25%			-2.63*				
	0–50%			-2.87*				
	0–100%	-3.76**						
	25–50%	-3.66**	3.61**					
	25–75%		3.54**		-2.77*			
	25–100%		-3.50**					
Within-subject effects	Year (Y)	5.24**	3.41*	15.40***	3.32*	18.19***	17.17***	14.32***
	Y×D0	23.72***	18.16***	9.04***	0.39	41.23***	40.63***	65.65***
	Y×EL	0.511	3.66*	3.51*	2.13	3.72*	1.71	3.59*
	Y×H	51.67***	2.46**	22.29***	10.79***	21.23***	24.07	18.65***
	Y×EL×H	0.5	1.495	2.38*	1.96	2.50*	2.32*	2.76**

Values presented in the table are test-statistics (F- and t-values for repeated ANCOVA and post-hoc test of harvest treatments, respectively). Levels of significance (*p*) are; ***<0.001, **<0.01, and *<0.05. Sampling plot was included as random (subject) factor. Further explanation on response variables are provided in **Table 6**, Appendix Table B.1–B.3 and Appendix Figure C.1–C.4.

Similar to ANCA, severely harvested subplots underwent a slower restoration process, which is slowed even more when it comes to populations in the higher alpine

(Table 7). Nevertheless, in Lauribina (4000 m), harvesting of 25% subplots brought up to increase of >1%, 20%, and 23% ramet density by 2016, 2017, and 2018 respectively, relative to the pre-harvest level, which indicates a possibility of regulated annual harvest (Table B.9, Figure 13). In fact, in lower alpine populations, the 50% harvest treatment exceeded pre-harvest density by 7% in 2018 (Table 6). Tukey post-hoc tests identified significant contrasts only between the control and the heaviest harvest treatment (75% and 100%), might be needed a wider interval of harvest levels to detect the variance (Table 7).

4.4.2 Effect of Harvesting on Vital Functions (Reproductive Activities) and Their Restoration

Harvest had significant effects not only on density but also on sexual reproduction (Table 7; Figure 15; Tables B.10–B.13; Figures C.3–C.4). Before harvest treatments were applied, we recorded a lower number of fruits per fruiting ramet in ANCA (5.45 ± 0.40) than in LNP (7.07 ± 0.59 , $p < 0.05$). We also recorded a difference between lower (6.56 ± 0.63) and upper (4.33 ± 0.40 , $p < 0.01$) alpine populations within the ANCA, whereas the number of fruits per fruiting ramet did not vary significantly within the LNP. In ANCA, we observed no sexual reproduction of ramets in upper alpine populations treated with harvest intensities >50% in both monitoring years, 2016 and 2017 (Figure 15). Within the control subplots, reproductive stage ramets in lower alpine populations invested almost twice as many resources in floral structures as those in upper alpine populations ($p < 0.01$). On average across the three years, we observed that 64% of floral structures developed into intact fruits in lower alpine populations (55%, 69%, and 71% for 2015, 2016, and 2017).

Fruiting abortion was always highest for upper alpine populations, where only 51%, 43%, and 51% of floral bodies developed into intact fruits in 2015, 2016, and 2017, respectively (Figure 15, Table A.12), but there was no significant variation in fruit abortion across elevations ($p > 0.05$, Table 7). However, a significant interaction between harvest and elevation indicated that harvest impact on fruit abortion varied between lower and upper alpine populations. In general, reproductive investment and fruit abortion showed an inverse relationship. The number of intact fruit, seed production, and fecundity were positively related to pre-harvest ramet density and negatively to harvest intensity and elevation (Table 7).

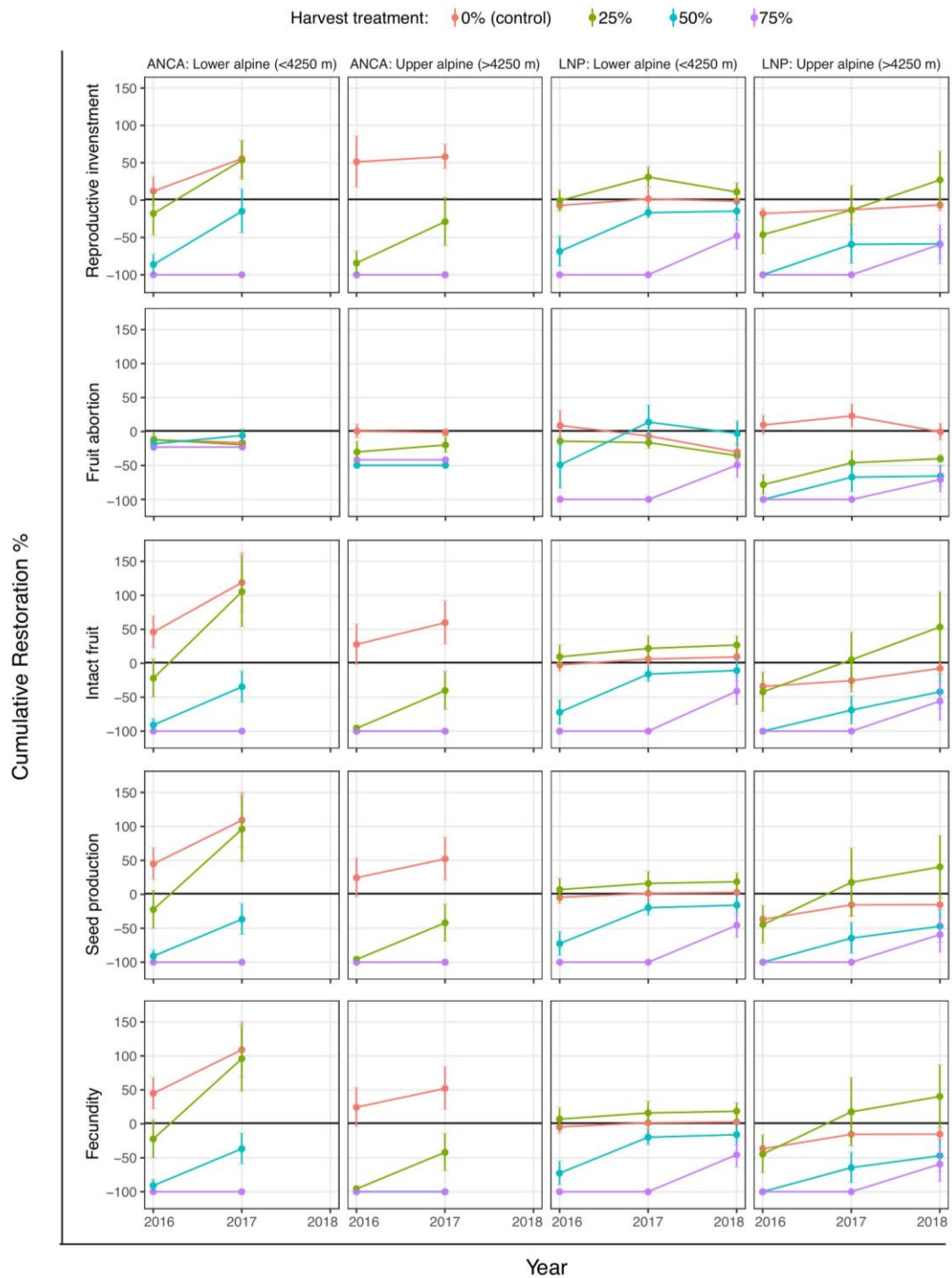


Figure 15: Relative change of mean (\pm SE in %) reproductive activities/traits [sequentially top to bottom: fruit abortion, intact fruit setting, reproductive investment (totaling all values: flower + bud + aborted/intact fruit), potential seed fecundity (seed number \times proportion of seedling established from seed), and seeds set] per ramet of *Neopicrorhiza scrophulariiflora* with respect to pretreated density (m^{-2}) at 2015 prior to apply harvest treatment in the same year. Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), underwent annual postharvest restoration monitoring for two years and three years, respectively. Further details on trait measures are provided in **Figure C.3–4**.

The pre-harvest ramet fecundity was 37% higher for lower alpine than for upper alpine plants ($p < 0.001$). Increasing harvest levels further decreased fecundity in upper alpine populations (**Figure 15**). Meanwhile, the time to maturity was an important factor in promoting fecundity, which compensated for higher levels of harvest, and for the negative effect of elevation ($p < 0.001$, **Table 7**). Also in LNP, observations made within the control subplots showed that plants invested more in sexual reproduction in lower than in higher alpine populations but the difference was smaller than in ANCA (**Figure 15**). On average 68% of the fruits turned out to be intact (65%, 69%, 67%, 72%; for 2015, 2016, 2017, and 2018), compared to an average of 55% for the upper alpine populations (59%, 49%, 53%, 61%; in 2015, 2016, 2017 and 2018, **Table B.13**). Also, production of fruits increased with pre-harvest density ($p < 0.01$), decreased with increasing harvest intensity ($p < 0.001$), and increased with elevation ($p < 0.01$). In harvested subplots, fruiting increased significantly over time ($p < 0.001$, **Table 7**). On average, 27–34% of floral structures were aborted in control subplots. This increased as harvest intensity rose ($p < 0.001$), but neither elevation nor pre-harvest density had any appreciable influence ($p > 0.05$). Intact fruit production per ramet was positively related to pre-harvest density ($p < 0.001$), but negatively with increasing harvest ($p < 0.001$) and elevation level ($p < 0.01$).

The time needed for restoration to the pre-harvest level increased with increasing pre-harvest density ($p < 0.001$), with treatment intensity ($p < 0.001$) and elevation ($p < 0.01$). However, seed production per ramet was not influenced significantly by elevation ($p > 0.05$), which might be needed to compensate for the reduced fruits along with the increasing negative effect of elevation towards upper alpine populations (**Figure 15, Table 7**). Comparing seed fecundity per ramet across control subplots it emerged that, on average, plants in LNP were 45% more fecund than plants in ANCA, and within the LNP over the same years, lower alpine populations were 68% more fecund than upper alpine populations ($p < 0.05$, **Table B.13**). Also in LNP, fecundity was positively associated with pre-harvest density ($p < 0.001$) and negatively with increasing harvest ($p < 0.001$). The fecundity almost recovered to the pre-harvest level within three years, both for lower and upper alpine populations, and for 25% harvest treatment the increase in fecundity was 18–40% (**Table B.13**). However, the restoration became lower and irregular for harvest treatments exceeding 50% (**Figure 15, Table 7, Table B.13**).

4.4.3 Interrelationships Between Ramet Mortality and Harvest

In both study sites, the highest number of ramets died for the 75% harvest treatment and in the first year of harvest, 2015–16 (**Tables B.10–B.12, Figure C.5**). In ANCA, mortality increased in subplots with high pre-harvest ramet density ($p < 0.05$) and higher harvest treatments with 50 to 100% extraction ($p < 0.001$) (**Table B.10**). However, it decreased over time in harvested subplots ($p < 0.001$). Although, mortality did not significantly vary with elevation ($p > 0.05$) alone, significant interaction effects of time, elevation, and harvest treatment ($p < 0.05$) indicate that mortality responded to particular combinations of these factors (**Table 7**). In LNP, mortality was highest in subplots where pre-harvest density was highest ($p < 0.001$), just as in ANCA. Also, higher harvest intensity led to higher mortality ($p < 0.05$) (**Table 7, Table B.11**). Unlike in ANCA, elevation had a clear positive impact on mortality ($p < 0.01$). But likely to be in ANCA, a significant interaction between time and harvest intensity ($p < 0.01$) and, time and elevation ($p < 0.05$) indicated that time duration was an important factor but a more complex combination might be required for lowering mortality when the harvest intensity was higher (**Figure C.5**).

4.5 Discussion

4.5.1 Factors Governing Sustainability

Ramet density restoration in *Neopicrorhiza* was driven by re-sprouting from old rhizomes and was strongly affected by harvest intensity, with higher harvest intensity leading to slower recovery. Our findings agreed with previous studies that high budding potential in clonal plants exposed to low harvest intensity enhances growth rates through vegetative cloning (Sanders and McGraw, 2005; Ghimire *et al.*, 2005; 2008). The clonal shoots may have opportunities to exploit nutrient-rich heterogeneous habitats offered by soil disturbance resulting from harvest, which improves aeration for newly recruiting ramets (Kindscher *et al.*, 2019). Typically, when a rhizome is removed, a sizable number of rhizome fragments with lateral roots may remain in the soil. Adventitious buds from these fragments may boost ramet density above pre-harvest levels or soon restore it. However, the trends in regrowth varied between study sites and populations at different elevations. Also, Poudeyal *et al.* (2019) observed that harvesters extract increasing quantities of rhizomes in ANCA and need to extract almost twice as many ramets as in LNP to achieve the same volume of *Neopicrorhiza*.

The latter observation shows that density measures focusing only on aerial parts are of limited value when deciding about management. Thus, we call for an annual assessment of biomass across a wide range of ecological conditions so that optimal harvest limits can be identified that are valid in each particular situation.

Mild disturbance has previously been reported to be positive for density growth in *Neopicrorhiza* (Ghimire *et al.*, 2005), and it is, therefore, possible that the mild disturbance caused by the tagging and monitoring activity every year enhanced the micro-environment and thus increased growth in our control subplots. For example, when tagging or measuring ramets, these might be pulled slightly upward, and neighboring plants of other species might be removed or turned away, and this could have improved ramets' access to light. Although our plots were in principle under control during the experiment, the overall slow recovery of *Neopicrorhiza* in ANCA populations could be linked to the fact that commercial harvest is legitimate in this area under current government regulations. In fact, a previous study reported that the ANCA landscape is anthropogenically disturbed by the heavy and partly premature harvest of *Neopicrorhiza* ramets (Poudeyal *et al.*, 2019). Sanders and McGraw (2005) indicated that premature harvest of the rhizomatous plant could reduce the carbohydrate flux available for regrowth at early and peak growing seasons relative to senescent times. Thus, ANCA populations might experience physiological stress for the recuperation of sufficient nutrients to regenerate new individuals. We therefore strongly recommend future work in nutrient dynamics analysis in harvested populations.

The present analysis focused on spatio-temporal response of plants to harvest intensities. In this analysis, we controlled for effects of site and elevation, which can be understood as proxies of environmental factors (e.g. precipitation, edaphic composition), but the basic environmental conditions in the Himalayas are changing concomitantly with the global rise in temperature (Rana *et al.*, 2020), presumably causing the locations of optimal habitats of plants to move. Previous studies have reported that in the long-term continuous harvest reduces the population sizes and allelic richness and, in this situation, plants may lose the ability to respond adaptively to a changing climate (Gaoue *et al.*, 2014; Rana *et al.*, 2020). To address this issue, we suggest conducting empirical research on genetic diversity and its development when a harvested population is subjected to a warming climate.

Harvesting has a negative impact on population stability because it disturbs intrinsic critical processes like reproductive investment, fruit production, seed set, and

plant regeneration potential (fecundity) (Ghimire *et al.*, 2008a; Kindscher *et al.*, 2019). The restoration of plant density to pre-harvest levels was found to be quicker than the restoration of the intrinsic vital functions in all treated plots up to the 50% harvest treatment. But, the complete loss of reproductive success of plants for all subplots subjected to 100% harvest and most subplots with 75% harvest showed that the vital functions are not resilient to highly intensive extraction. However, in subplots with 25% harvest, the increase in capacity of setting fruit, seed production, and unchanged or improved fecundity, compared to the pre-harvest situation (in most of the cases) indicated that the vital functions of the plants are fairly resilient to low intensities of ramet removal.

However, in the case of upper alpine populations, inferior seed fecundity and slow recovery showed that caution should be exercised when applying intensive harvesting. This finding is consistent with a previous study showing that harvest reduces seed setting and fecundity of rhizomatous plants (Ghimire *et al.*, 2008a). In conjunction with the effects of harvest, low temperature, unstable weather, and a short growing season caused a stressful situation for the plants (Poudeyal and Ghimire, 2011; Rana *et al.*, 2020).

4.5.2 Sustainable Extraction Based on Experimental Harvesting

Our study showed that 25% extraction of *Neopicrorhiza* was restored in most conditions, with two years recovery period. An exception was observed for populations located above 4500 m. By contrast, for lower alpine populations in LNP, the density growth and reproductive activity of subplots with 25% harvest intensity exceeded those of the control plots. In the lower alpine populations, harvest was acceptable up to 50% extraction and the density had totally recovered to pre-harvest levels after three years. Nevertheless, populations at elevations >4250 m did not recover to pre-harvest levels within the observation period, likely indicating a population decline. Harvest levels >75% totally nullified the reproductive capacity of upper alpine populations in both ANCA and LNP within the duration of the monitoring period, possibly because the rhizome reserves were insufficient for reproduction. A similar pattern was observed for *Ligusticum porteri* by Kindscher *et al.* (2019), where the effects of high levels of harvest on vital functional traits were unrepairable. The larger differences between the experimental harvest intensity levels in ANCA than in LNP showed how our one-time harvest treatment interacted with the annual disturbances in the open-access site

compared to the undisturbed, restricted-access site. Thus, we propose regulating extraction through a rotational management system, where the time between subsequent harvest events is at least two years for 25% harvest and four years for 50% harvest intensity. At higher elevations (>4250 m) we suggest lengthening the rotations by one year for both harvest intensities to ensure sustainable extraction and viable recovery.

4.5.3 Implications for Sustainable Management and Conservation

Harvest per se is not a problem for the sustainable management of *Neopicrorhiza* populations, but overharvest is. With subplot size applied in our harvest experiment, regeneration occurs even with 100% harvest treatment, although the rate was very slow. This suggests that sustainability is possible if rotational harvest is implemented and sufficiently long time intervals are allowed for sexual reproduction. Both study sites are located in protected areas, but present conservation measures are unable to address the ever-increasing pressure of commercial collection — in our study sites particularly in ANCA, but also as reported in many other protected sites in the Himalayas (Ghimire *et al.*, 2008b; Uniyal *et al.*, 2011; Manish and Pandit, 2019). Evidence indicates that *Neopicrorhiza* has a good potential for cultivation (Ghimire *et al.*, 2005; Chandra *et al.*, 2006). Our ongoing research of *in situ* transplantation experiment at the plot established in LNP (4200 m) provided that up to 85% of adult ramets remain to survive to next year's observation and up to 50%, of new ramets, added later than two years. However, so far, no attempts to cultivate the species have been successful at a lower elevation than its native distribution range which led to organized cultivation on a larger and commercial scale (Chandra *et al.*, 2006). So, we recommend establishing long-term *in situ* transplantation experiments in a wider environment to collect scientific evidence prior to launching cultivation programs. Overall, this suggests that the development of *in situ* cultivation extensions and proper harvest strategies for the habitat management of wild populations remains the basis for sustainable conservation.

4.6 Conclusion

Neopicrorhiza populations can quickly recover from low-intensity harvest. With our particular methods, growth appears even better for lightly harvested subplots than for unharvested subplots, both for density and reproductive activities. Based on the

observed density restoration, we recommend up to 25% for upper alpine and up to 50% extraction for lower alpine populations at intervals of four years. Recovery appears to be slower in the site commercially open for harvest and in upper alpine populations at elevations greater than 4250 m. Thus caution is needed when implementing harvesting programs in these areas. Also, we conclude that harvest levels involving the removal of more than 50% of the ramets lead to such a slow recovery that it cannot be described as sustainable. Hence, we strongly recommend introducing spatiotemporal harvest regulation measures for long-term conservation and maintenance of the resource as a basis for income generation. Further, quantification of the transient dynamics of plant life-stage classes, their growth rates, and the effects of harvest are necessary as a basis for sustainable management.

CHAPTER 5

5. POPULATION DYNAMICS AND SUSTAINABLE HARVESTING¹

5.1 Abstract

Globally, the wild harvest of medicinal and aromatic plants (MAPs) driven by commercial demand causes serious ecological concerns for resource depletion. Harvest-driven ecological impacts and approaches to mitigate these impacts can be observed elsewhere; however, management systems are often ineffective due to the lack of consolidated field-based evidence. To bridge this gap, we collected data on regeneration, recruitment, fecundity, growth, and survival of the commercialized clonal herb *Neopicrorhiza scrophulariiflora*, which is distributed in subalpine and alpine areas of the Himalayas, and was studied in open-access and protected areas in north-western and -central Nepal. We used stage-based annual (2015-2018) population transition matrices and *in situ* experimental harvesting treatments to explore plant responses and the fate of populations and to find optimal harvest limits. Generally, a lower level of harvesting induces vegetative sprouting. For up to 25% removal of plants, the growth rate corresponded to the pre-harvest condition for a 2-years harvest interval in almost all studied populations. In contrast to this, the growth rate was retrograded even in the control treatment, and harvesting became further deteriorative to populations located in the upper alpine open-access area, whereas for the protected area, removal of up to 50% did not affect population growth for the same transition period, instead, the growth rate escalated more than in control experiments. For sustainability, we propose spatio-temporal harvesting approaches and harvest intervals of at least 4 years so that plants can have sufficient recruitment and adequate viability. Findings can be pivotal to the decision-making for sustainable harvesting and management of threatened Himalayan MAPs.

Keywords: Medicinal plants; recruitment; matrix modelling; over-exploitation; sustainability; alpine Himalaya

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5.2 Introduction

The demand for wild-harvested medicinal and aromatic plants (MAPs) has recently been surging as a consequence of the rapid increase in economic growth, high purchasing capacity, new trends in nutrition, and emerging herbal markets, particularly in developed countries. However, the *in situ* management and sustainable harvest of MAPs, which are supplied mainly by developing countries, is a topical ecological issue elsewhere in the world (Schippmann *et al.*, 2006). Moreover, for sustainable resource exploitation and supply, harvest-driven ecological consequences such as a negative impact on regeneration potential and demographic maintenance are less appreciated and poorly documented, particularly for MAPs that are harvested from remote mountains and subjected to increasing commercialization (Huai *et al.*, 2013; Rokaya *et al.*, 2017).

Nevertheless, harvesting and trade of wild plant resources including MAPs is a pervasive livelihood-supporting tool for primary health care and as a cash income-generating source through commercialization in most rural parts of the world. Besides, economic incentives generated through trading of excess amounts and cautious harvest of forest products is considered key contributing parameter that can be implemented for the promotion of stewardship and management extension programs, such as for cultivation and other *ex situ* conservation activities (Pyhälä *et al.*, 2006). At present, several paradigms for sustainable harvesting of MAPs are available, where plant populations are managed *in situ* and well-conserved in their natural condition; and side-by-side improving the economic status of local livelihoods (Schmidt *et al.*, 2019). Judicious removal of old, dried, and broken branches, and excess biomass of plants may help to divert energy resources to productive sites; distribute the light to all green plant parts; reduce the potential mechanical injury caused by harvest; maintain the root-shoot ratio; and ultimately improve the productivity. Such regenerated plants after harvest may increase their vigor of growth such as the quick arrival of meristematic tissue; more leaves, fruits, and seeds.

In general, in mountain areas such as the Himalayas, harvesting MAPs is often a selective and well-considered activity where traditional users consider the potential regeneration and future availability, whereas commercial harvesters identify and harvest the most valuable individuals and often choose those of larger size (Ghimire *et al.*, 2004). When harvest aims to remove major portions or whole parts of the plants it

may be destructive and lead to uncertainties with regard to demographic processes, such as mortality of remaining individuals; deceleration of fecundity and reproduction; and retardation of survival, growth, regeneration, and recruitment (Ticktin, 2004; Chen *et al.*, 2015). Ultimately, such plant populations affected by increased isolation, degrade the evolutionary potential and individual fitness and can be driven to the brink of extinction. However, most of the existing harvesting practices applied to MAPs in developing countries like Nepal, are regrettably said to be inefficient and wasteful, and almost all harvest recommendations at the policy level lack empirical evidence for estimating sustainable rates of extraction.

Identification of a sustainable rate of extraction and collection of demographic data for harvested plant populations are arduous tasks but globally appreciated to make conservation decisions, particularly for wild-harvested and threatened MAPs (Small and Chamberlain, 2018; Schmidt *et al.*, 2019). Over the last three decades, matrix modelling has become a well-known approach used in determining transient dynamics of the population over time, whereby plant individuals are divided into discrete classes based on stage or state forms (Caswell, 2001; Schmidt *et al.*, 2011). In this context, the stage of development is described on the basis of above-ground structures, the presence of an upright stem, the presence of flowers, etc. and alternatively described based on the measures of size, such as the number of leaves, height, length of leaves, or a combination of size and presence of particular structures. Each class of individuals has a distinct survival rate and fecundity contribution to the next generation. Vital rates (survival, growth, and fecundity) of each stage class are repeatedly measured to build matrices. Further, the application of models to trade-related MAPs provide population responses across different harvest types (intensity and frequency) and shows whether demographic growth rates are increasing, remain stable or are declining (Ghimire *et al.*, 2008a; Schmidt *et al.*, 2011). More than that, matrix-based elasticity analysis can be used to suggest harvest strategies that enable maintaining or increasing population sizes even if the actual harvest intensity is characterized by uncertainty (Schmidt *et al.*, 2011). Particularly in Himalayan MAPs, the rigorous monitoring of ecological consequences of harvesting on populations under different environmental conditions needed to develop a standardized method is only to a limited extent available, which hampers habitat management programs.

Thus, we used annual (2015-2018) stage-based population transition matrices to estimate the fate of different demographic stages of the commercially harvested clonal herb *Neopicrorhiza scrophulariiflora* from the Nepalese Himalayas. We hypothesize that all levels of harvest may not be detrimental to the plant's fecundity, regeneration, and recruitment; and spatial and temporal response to plant extraction may determine the fate of populations and optimal harvest limits. To test this hypothesis, we asked the following questions: (1) In what ways does the harvest level affect fecundity, regeneration, and recruitment of *N. scrophulariiflora* populations? (2) How do population growth parameters (progression, retrogression, stasis, and death) interact with harvest level, time of recovery, and elevation gradient? (3) Do projected population growth rates (λ) vary with increasing harvest levels for plants growing in different environments? Which vital rates have the greatest impact on λ ? (4) What are the optimal harvest regimes and limits to sustainable resource extraction that can be reconciled with ecological integrity? The findings can be crucial for conceiving adaptive measures for sustainable harvesting and pivotal as a decision-making tool for the sustainable supply of threatened Himalayan MAPs.

5.3 Materials and Methods

5.3.1 Species Description

Neopicrorhiza scrophulariiflora (Scrophulariaceae, hereafter *Neopicrorhiza*), is a long-lived clonal herb. The global distribution is limited to Nepal, Tibet, Western China, Sikkim, and Bhutan in the narrow zone elevation between 2800 and 5000 m above sea level; and mostly restricted to cool north-facing slopes. Traditionally, the plant is used for the treatment of cough, cold, fever, liver diseases, intestinal disorders, poisoning, and many more, primarily supporting the Himalayan rural health systems. Pharmacological evidence shows that high quantities of iridoid glycosides are valued for hepato-protective, antidiabetic, and cancer treatment; however, extraction of such chemicals through increasing industrial use is threatening natural habitats. The harvest and trade have occurred since time immemorial, every year thousands of people are involved in the harvest and trade of MAPs from different parts of the Himalayas. Annually, about 66% of the global supply (175–770 tons of air-dried rhizome per annum) of *Neopicrorhiza* is extracted from the Nepalese Himalayas (Olsen, 2005).

Immature harvest and overharvest to meet the rising global demand and escalating prices pose a greater risk of local depletion (Poudeyal *et al.*, 2021).

The plant is clonal and polycarpic, possesses a prolonged (3–5 years) vegetative stage compared to the reproductive stage (2–5 months), and remains green under the snow throughout the winter. The seed germination and development of reproductive shoots may depend on climatic cues such as precipitation (140–160 mm) and adequate temperatures (10–13 °C), sometimes sprouts are relatively earlier if the spring is supported by frequent rainfall (Poudeyal *et al.*, 2021). The fully-grown mature ramet (physiologically connected shoot which has the potential to exist independently) has numerous dark-green leaves (>8 leaves in fully grown adult vegetative stage). The ground attachment of ramets to the rhizome is supported by earlier years' dead leaves, which may persist for more than a year and are linked to biophysical protection, e.g. limiting the risk of dehydration. A well-developed rhizome is up to 25 mm in girth, serves as a storage organ, and bears numerous deeply penetrating slender roots, reaching a depth of up to 15 cm (Poudeyal *et al.*, 2019). The ramification of ramets occurs from the axillary meristem at the nodal region of the rhizome. In the reproductive year, starting by June, when the size of an individual ramet exceeds a vegetative threshold period (2–4 years) it produces usually an erect terminal inflorescence with densely packed protandrous flowers. The majority of the flowers (>50%) produce fruits; each ramet bears 4–12 fruits and each fruit bears 20–60 seeds; however, the number of fruit and seed, and their firmness decreased with increasing elevation (Poudeyal *et al.*, in preparation). Seeds possess a thin transparent wing as an extra circular growth, which supports dispersal with air-current. The plant is regenerated with both seeder and adult ramets; seedling recruitment is very low (>1%), and seeds totally lose viability within a year (Ghimire *et al.*, 2005). However, to a large extent, the regeneration, establishment, and expansion of plant populations occur through vegetative cloning.

5.3.2 Study Area and Population Selection

In 2014, we conducted a reconnaissance visit to two locations in the Nepal Himalaya: Api-Nampa Conservation (ANCA), Darchula, and Langtang National Park (LNP), Rasuwa district, to gather data on the species distribution and exploitation. Legally, both ANCA and LNP belong to the protected areas of Nepal, established in 2010 and

1976, respectively. LNP is strictly protected for commercial harvest of environmental products, but in ANCA, conservation and management programs are based on local requirements, and the commercial harvest of MAPs constitutes the mainstay economy to the local people, and the area is thus a de facto open-access area.

Based on previous years' findings, we made a full-fledged survey in 2015 by selecting four populations along the catchment area of the Trisuli river in LNP (location: 28.09°N and 85.42°E), north-central Nepal. Along the elevation gradient, the populations were Lauribina (4000 m), Lauribina Pass (4200 m), Gosainkunda (4500 m), and Dudhakunda (4700 m). The next four were in the upper Chamelia river valley in ANCA (29.97°N and 80.96°E), northwestern Nepal, and located at Thadapani (3800 m), Thadeula (4200 m), Bainsad (4300 m) and Channi (4600 m). At both sites, the northern geographical boundary is the Tibetan border of Nepal to China and in addition to that, on the western side, ANCA borders Uttarakhand, India. Historically, both study sites are considered reservoirs of large numbers of MAPs directly linked to local use and trade. Unpublished records from ANCA and LNP show that 70 and 91 MAPs are listed in ANCA and LNP, respectively, and the majority of them are harvested for local use, and at both sites about 40–60% of them are harvested annually for trade.

Both sites belong to the historically popular rangeland for summer grazing of ungulate livestock. In recent years at ANCA, the collection of medicinal caterpillar fungus yatsa-gombo (*Ophiocordyceps sinensis*) supplements cash incomes in the rural economy. However, local people, particularly herders, are engaged in harvesting tradable MAPs due to low agricultural production and limited economic opportunities. Whereas in LNP, besides the tourism-based hotel business, herders are engaged in cow-milk production, which provides supplies for local dairy industries and reduces the pressure for MAPs exploitation.

Ecologically, the *Neopicrorhiza* is distributed from 3400–4600 m in ANCA and 3800–4700 m in LNP, mostly in shrubland meadows dominated by *Rhododendron*. The distribution is affected by the cardinal direction of the mountains, mostly towards the warm-northern slopes of the terrain. The selected populations were distinctly fragmented and very patchily distributed, and the range within which the species was found was estimated to be at least two kilometers of aerial distance. The density varied depending on plant community, topography, and land use type, about 20–500 individual ramets per square meter were recorded, and thus extrapolation to the total number of

individuals in a population could be overestimated. Due to wide environmental variation, populations are distinctly different from each other with regard to vital attributes such as clonal propagation, seed germination rate, establishment, and reproductive timing, which is directly influenced by the elevational gradient (Poudeyal *et al.* 2021). Thus, based on the elevation level two populations were considered as lower (<4250 m) and the other two were described as upper alpine populations (>4200 m) in each study site.

5.3.3 Field Measurement and Plot Establishment

The stage-based population projection matrix model was used to estimate the population growth parameters. In each population, three equal-sized (3×3 m) permanent plots were purposively established. Plot locations were selected based on homogeneity in density, that populations had not been harvested for at least five years, and that they were located at relatively secure sites in relation to human mobility and harvest. The plots were further divided into nine equal-sized (1×1 m) subplots to facilitate the relocation of tagged plants. Of the nine subplots, four at corners and one at the middle were picked for a thorough census. At each site, we established six plots (three per population) both in the lower alpine and upper alpine areas. Thus, we examined 24 plots (12 at each site) and a total of 120 subplots in the two sites.

Based on the number and size of leaves and existence of inflorescence, we divided plants in their vegetative and reproductive stages into four categories: (1) Sprouting (SP: Recently appeared shoot, either from seed or from the branching of already existing vegetative ramets, which can be considered to be potential clonal sprouters or regenerators), (2) Juvenile (JV: Juvenile vegetative young ramet, with 7 leaves or less and leaf size greater than or equal to 1.5 cm), (3) Adult vegetative (AV: Adult vegetative mature ramet, bearing more than 7 leaves and with largest leaves exceeding 2 cm in length), and (4) Reproductive ramet (Rep: Sexual reproductive ramet with flowering/fruitlet peduncle; the next stage after AV). The transitional vegetative stage between sproutings and flowering individuals we carefully reclassified into two distinct classes (JV and AV), implying that these two stages cover a fairly long period of time and there would be a high probability of no change for this class from year to year. As a result, highly dissimilar individuals were distinguished, which improved the representation of the transient dynamics of the population. Each stage was considered

to have a distinct set of parameters, including mortality, fecundity, and ontogenetic stage transition rates (Easterling *et al.*, 2000). In each annual census, we noted leaf numbers, their dimension for up to three largest leaves, the presence of inflorescence, and the state of each ramet.

5.3.4 Harvest Treatment and Monitoring

In 2015, we thoroughly recorded the preharvest density and determined the number of ramets to be harvested in each subplot. We took the prior consent of local harvesters in each experimental site and requested them to mimic their regular harvesting procedures in our experimental subplots. One subplot from each of the five measured subplots served as the unharvested control (0% harvest), while the other four were randomly assigned to one of four experimental harvest treatments: 25%, 50%, 75%, and 100% removal of ramets, regardless of the plant size. For 100% harvest, we removed all the ramets contributing to the surface cover, but we were unable to remove small rhizome fragments in the soil that could be a potential source of postharvest regeneration in each treatment (Small and Chamberlain, 2018). We developed six replicas (three in each population) of the harvest treatments, each in the lower and upper alpine areas.

After the harvest, all remaining plants in each subplot were labeled and mapped. Each corner of the plots was marked with elongated rocks stuck into the ground and an aluminum tag with an embossed number was placed at each ramet. The tag was tied to a bamboo peg (10–15 cm) with the help of a twisted electric wire. If ramets were too close to the tags of other ramets, distances, and directions from each bamboo peg to the corresponding ramet were noted, thereby helping to identify individuals. Each subplot was visited and censused annually in the months of July-August until 2017 for ANCA and 2018 for LNP. Thus, in total, we observed two transitions for the former and three for the latter. At each visit, newly arrived individuals were minutely searched for, and newly discovered ramets were tagged.

5.3.5 Vegetative and Sexual Fecundity, Regeneration, and Recruitment

Both regeneration strategies (vegetative/clonal and sexual/fruitleting) of the plant were thoroughly observed and recorded. During the fruit/seed maturity season in September 2015, 30 fruiting individuals from various genets (source plants) were randomly chosen from each population at a distance of at least 5 m from the density plots. Five healthy,

fully matured fruits per ramet were picked, and the number of seeds per fruit was calculated. Seeds were packed in filter paper and filter paper pockets were kept in muslin mesh bags and stored in a rocky cave near the habitat of the mother population. At the beginning of the next growing season June (2016), we introduced a germination experiment using fully matured seeds ($n = 200$, seeds from five fruits) in well-prepared seedbeds at identical sites in the vicinity of the mother populations where *Neopicrorhiza* were absent. For each population, the *in situ* seed germination experiment was conducted three times in 1-by-1-meter beds. The number of seedlings recruited in each seed bed was monitored annually up to 2018 for all the populations. These germination and recruitment experiment results, combined with data on the number of seeds produced by each fruit or plant, were utilized to compute the reproductive individuals' fecundities (Ghimire *et al.*, 2008a). Seedling recruitment proportions were multiplied with seed number per ramet in each subplot to estimate the sexual fecundity. As stated by Ghimire *et al.* (2005; 2008), the seeds rapidly lose viability (%) after a year, and therefore seed cohort was not considered in the matrix construction.

In addition to the fecundity resulting in seedlings regenerating from sexual reproduction, vegetative fecundity also appeared meaningful to the population growth rates. Further, our five-year (2014–2018) field observation showed that newly sprouted ramets never propagated through either vegetative or sexual (flower/fruit production) reproduction in the same year, and ramets might need several years to reach the flowering stage. Moreover, since sexually reproducing individuals never sprouted to form vegetative clones in the same season, resource acquisition time might be too long to achieve both types of reproduction in the same season. Thus, along with seed germination-based fecundity for flowering individuals, we also introduced separate estimates of vegetative fecundity for the Juvenile and Adult vegetative stages (Berg, 2002). In each subplot, sprouts produced by Juveniles and Adults were counted thoroughly in each annual census. Later, the number of sprouts for each stage and in each subplot was divided by the number of ramets in the respective stage classes to estimate the fecundity of each, considering them as potential sprouters (Berg, 2002). The total number of sprouts in each subplot was divided by the number of potential sprouters from the previous year's count to get the regeneration percentage. The

regeneration percentage includes the new or old ramets that may have the probability of death.

We used the formula stated by Pandey and Shackleton (2012) to calculate the recruitment percentage of the ramets in each subplot:

$$\frac{1}{\text{Time in year}} \times \ln \left(\frac{\text{Sprouter} - \text{Dead ramet} + \text{Sprout}}{\text{Sprouter} - \text{Dead ramet}} \right) \times 100\%$$

The dead ramets which had no indication of life in the aerial parts and had no probability of regeneration again were totally eliminated from the recruitment percentage. We assumed that exponential recruitment occurred in each of the experimental plot and over a 1-year period, 100% old ramets (potential sprouters) estimated to be producing new ramet (sprout).

5.3.6 Matrix Construction and Relationship Between Demographic Growth and Environmental Factors

Demographic parameters were calculated using stage-based population projection matrix models (Caswell, 2001; Ticktin *et al.*, 2002; Ghimire *et al.*, 2008a). Two different kinds of transition matrices were created: (1) annual matrices, which were based on data from each annual census, and (2) total matrices, which were created by averaging the class-specific individual number values over all of the population's matrices for the year. The plant size and state categories (**Table 8**) were combined to classify individuals into four stage classes. Following the fate of stage-classified plants over a one-year period allowed us to create the annual transition matrices for each population and treatment (Berg, 2002). Elements F_{12} , F_{13} , and F_{14} of the matrix's first row correspond to the fecundity values, which show the average contributions of individuals in the reproductive categories to the seedling class. As juveniles (JV) and adult vegetative (AV) individuals also reproduce to some extent through the vegetative method. The average number of sproutings/seedlings generated per reproductive adult (F, fecundity); percentage of survivors who remained in the same size class (S, stasis); percentage of survivors who expanded to a bigger size class (P, progression); and percentage of survivors who shrank into a smaller size class (R, retrogression) were used to construct annual transition matrices. The Popbio tool in the R statistical software program was used to examine each transition matrix and determine the

asymptotic population growth rate (λ) (Stubben and Milligan, 2007; R version 4.1.0: R Core Team, 2021). If >1 , the population grows and is deemed viable; if $\lambda < 1$, the population drops and is deemed unviable; and if $= 0$, the population stays at a steady equilibrium density. Using Keyfitz's dissimilarity index, the differences between the observed and stable stage distributions were evaluated (Keyfitz, 1968; Ghimire *et al.*, 2008a). We made sensitivity and elasticity analyses to identify which stage classes may have the highest impact on λ and how this was affected by harvest.

We used a linear mixed effects model to estimate the effect of predictors (harvesting treatment, transition year, and elevation) of fecundity, regeneration, and recruitment; and to check the strength of predictions of those independent variables among the ecological regions. The sample plot was included as a random factor and the lmerTest package was used in preparing linear mixed-effect models. Similarly, the effects of harvest and environmental variables on the demographic parameters; progression, retrogression, stasis, and mortality were analyzed through logistic regression to detect positive and negative impacts; and their variability among the ecological regions. In order to do this, we employed generalized linear modeling (glm) using a binomial distribution and a logit-link function for each response variable (Crawley, 2007; R Core Team, 2021).

Table 8: Matrices of state vector transition probabilities based on the distribution of the plant's stage categories with potential stage transition probabilities from one year (t, columns) to the next (t + 1, rows). Matrix elements are noted as stasis (S), progression (P), retrogression (R) and fecundity (F)

	Ramet stage	Year (t)			
		SR	JV	AV	Rep
Year (t+1)	Sprouting (SR)	-	F ₁₂	F ₁₃	F ₁₄
	Juvenile vegetative (JV)	P ₂₁	S ₂₂	R ₂₃	-
	Adult vegetative (AV)	P ₃₁	P ₃₂	S ₃₃	R ₃₄
	Reproductive (Rep)	-	P ₄₂	P ₄₃	-

5.4 Results

5.4.1 Fecundity, Regeneration and Recruitment

At both ANCA and LNP, the vegetative fecundity, annual regeneration, and recruitment percentages were higher in lower alpine populations in control conditions (**Table 9**). The manually calculated average sexual fecundity per ramet was 0.0038 in ANCA and

0.0063 in LNP. The preharvest density was relatively lower for upper alpine populations compared to lower alpine in both study sites. A very low percentage of ramets were regenerated (vegetatively) and recruited for >50% harvest treatment. In every treatment, a higher number of ramets were regenerated and recruited in the first year (2016) than in the following years (**Table 9**). This trend appeared even in the control subplots and the harvest treatment had a positive impact ($p<0.05$) on the arrival and establishment of new ramets. In the lower alpine area of ANCA, a significantly ($p<0.05$) higher number of plants regenerated and recruited for subplots treated with 25% removal of ramets. While in the LNP, regeneration, and recruitment percentage was higher for the subplots treated with 50% removal of ramets in almost all years included in this study (**Table 9**).

Table 9: Vegetative fecundity, regeneration and recruitment status of *Neopicrorhiza scrophulariiflora* in the Api-Nampa Conservation Area and Langtang National Park, Nepal, along the experimental harvest treatments and elevation gradient

Response variables	Year	Lower alpine (<4250 m)					Upper alpine (>4250 m)				
		0%	25%	50%	75%	100%	0%	25%	50%	75%	100%
Api-Nampa Conservation Area (ANCA), north-west Nepal											
Preharvest density /m ²	2015	68 ± 10.16	56.67 ± 12.61	105.00 ± 19.53	176.67 ± 37.03	99.83 ± 19.59	54.67 ± 10	36.83 ± 6.24	64.00 ± 17.32	102.67 ± 30.12	110.17 ± 18.7
	2016	0.66 ± 0.21 ^a	0.92 ± 0.24 ^a	0.38 ± 0.07 ^a	0.47 ± 0.05 ^a	-	0.38 ± 0.10 ^a	0.88 ± 0.20 ^a	0.94 ± 0.25 ^a	0.83 ± 0.24 ^a	-
Vegetative fecundity/m ²	2017	0.35 ± 0.07 ^a	0.64 ± 0.24 ^a	0.47 ± 0.18 ^a	0.67 ± 0.18 ^a	-	0.28 ± 0.07 ^a	0.66 ± 0.11 ^a	1.21 ± 0.40 ^a	0.91 ± 0.29 ^a	-
	2016	14.71 ± 1.99 ^a	23.87 ± 6.24^{ab}	7.76 ± 1.74 ^c	3.70 ± 0.85 ^{cd}	11.24 ± 1.59 ^{abcd}	11.69 ± 3.92 ^a	17.21 ± 2.69 ^a	18.15 ± 5.5^a	9.36 ± 2.59 ^a	9.19 ± 1.60 ^a
Regeneration %	2017	15.44 ± 3.15 ^a	20.90 ± 6.97^{ab}	10.36 ± 3.00 ^{abc}	5.57 ± 1.12 ^{cd}	8.27 ± 1.81 ^{abc}	11.77 ± 2.66 ^a	22.49 ± 3.42^{ab}	20.93 ± 4.85 ^{abc}	7.16 ± 1.79 ^{ad}	6.87 ± 1.20 ^{ade}
	2016	14.24 ± 1.70 ^a	22.31 ± 5.09^{ab}	8.03 ± 1.74 ^c	4.02 ± 0.89 ^{cd}	-	11.20 ± 3.51 ^a	16.71 ± 2.57 ^a	19.85 ± 6.05^a	9.70 ± 2.58 ^a	-
Recruitment %	2017	7.38 ± 1.34 ^a	9.74 ± 3.07^{ab}	5.18 ± 1.44 ^{abc}	2.79 ± 0.55 ^{cd}	4.15 ± 0.88 ^{abcd}	5.63 ± 1.19 ^a	11.54 ± 1.64^b	10.68 ± 2.51 ^{abc}	3.91 ± 0.99 ^{ad}	3.45 ± 0.59 ^{ade}
	Langtang National Park (LNP), north-central Nepal										
Preharvest density/m ²	2015	91.00 ± 10.83	52.00 ± 10.35	55.67 ± 13.42	90.67 ± 22.92	100.33 ± 21.85	84.67 ± 15.37	40.50 ± 8.91	67.33 ± 18.15	162.67 ± 21.23	118.25 ± 32.96
	2016	0.37 ± 0.06 ^a	0.85 ± 0.30 ^{ab}	1.53 ± 0.23 ^{bc}	1.15 ± 0.41 ^{bcd}	-	0.22 ± 0.03 ^a	0.57 ± 0.09 ^{ab}	0.90 ± 0.18 ^{bc}	1.30 ± 0.21 ^{bcd}	-
Vegetative fecundity/m ²	2017	0.27 ± 0.03 ^a	1.58 ± 0.70 ^b	2.7 ± 1.10 ^{bc}	1.24 ± 0.21 ^{bcd}	-	0.18 ± 0.02 ^a	0.41 ± 0.09 ^{ab}	1.11 ± 0.29 ^c	0.75 ± 0.10 ^{bcd}	-
	2018	0.17 ± 0.03 ^a	0.28 ± 0.05 ^{ab}	1.07 ± 0.23 ^c	0.77 ± 0.17 ^{bcd}	1.52 ± 0.23 ^{cde}	0.13 ± 0.02 ^a	0.43 ± 0.09 ^b	0.7 ± 0.17 ^{bc}	0.23 ± 0.04 ^{abd}	1.22 ± 0.13 ^{bcd}
Regeneration %	2016	14.21 ± 1.67 ^a	25.99 ± 8.53 ^{ab}	26.60 ± 4.5^{abc}	8.68 ± 2.06 ^{ad}	10.48 ± 2.50 ^{ade}	10.86 ± 1.12 ^a	18.22 ± 2.97 ^a	18.37 ± 2.94^a	14.60 ± 2.39 ^a	12.28 ± 4.22 ^a
	2017	11.90 ± 1.81 ^a	36.20 ± 11.17 ^b	37.61 ± 7.21^{bc}	10.12 ± 1.72 ^{ad}	9.68 ± 3.06 ^{ade}	8.71 ± 1.02 ^a	14.22 ± 2.60 ^{ab}	24.80 ± 5.23^{bc}	6.14 ± 0.82 ^{ad}	9.83 ± 0.96 ^{abcd}
	2018	9.66 ± 1.64 ^a	14.02 ± 1.21 ^{ab}	28.17 ± 4.74^{bc}	8.31 ± 1.52 ^{abd}	6.73 ± 2.13 ^{ade}	7.92 ± 0.83 ^a	18.74 ± 3.89^{ab}	18.33 ± 2.93 ^{bc}	3.12 ± 0.40 ^{ad}	7.20 ± 2.15 ^{abde}
Recruitment %	2016	14.17 ± 1.62 ^a	24.88 ± 8.03 ^{ab}	26.25 ± 3.89^{bc}	9.15 ± 2.09 ^{ab}	-	10.40 ± 0.99 ^a	17.60 ± 2.77 ^b	18.43 ± 2.79^{bc}	15.06 ± 2.31 ^{abc}	-
	2017	5.95 ± 0.88 ^a	15.88 ± 3.90 ^b	17.06 ± 2.72^{bc}	5.38 ± 0.95 ^{ad}	4.76 ± 1.46 ^{ade}	4.49 ± 0.55 ^b	6.97 ± 1.17 ^{ab}	11.46 ± 2.16^{bc}	3.18 ± 0.44 ^{ad}	4.98 ± 0.36 ^{abde}

2018	3.31 ± 0.55 ^a	4.84 ± 0.45 ^{ab}	9.42 ± 1.49^{bc}	2.91 ± 0.55 ^{ad}	2.41 ± 0.79 ^{ade}	2.74 ± 0.32 ^a	6.11 ± 1.11 ^{ab}	6.20 ± 1.04^b	1.06 ± 0.14 ^{ad}	2.50 ± 0.76 ^{abd}
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The provided mean values in each row with different letter varied significantly at $p < 0.05$.

The vegetative fecundity, regeneration, and recruitment showed a negative relationship with the harvest treatment in ANCA, however, the case was almost reversed in LNP, and harvest showed positive to those parameters (**Table 10**). Also, in the meantime, the rate of recruitment decreases with the increasing of year in ANCA, otherwise, effects of elevation and time (number of years) were non-significant to vegetative fecundity and regeneration at both sites. However, the combined effect of harvest and elevation both with regeneration and recruitment was contradicting, negative in LNP and positive in ANCA (**Table 10**). The time alone was negative to the recruitment percentage in ANCA and invaried in LNP and interestingly, combining time and harvest treatment became unnoticeable in ANCA. Despite that in LNP, both regeneration and recruitment percentages reduced ($p < 0.001$) when the combined effect of time and harvest interacted with each other (**Table 10**).

Table 10: Linear mixed effect model of *Neopicrorhiza scrophulariiflora* to examine the effect of harvest treatment, time (year) and elevation on vegetative fecundity, regeneration, and recruitment rate per m² at Api-Nampa Conservation Area and Langtang National Park, Himalaya Nepal

	Vegetative fecundity		Regeneration percentage		Recruitment rate	
	t value	Pr(> t)	t value	Pr(> t)	t value	Pr(> t)
Api-Nampa Conservation Area						
Intercept	1.676	0.1	9.967	<0.001***	13.609	<0.001***
Harvest treatment percent (HTP)	-2.711	0.008**	-2.547	0.013*	-2.827	0.006**
Transition year (T)	-1.794	0.076	0.336	0.738	-3.334	0.001**
Elevation (E) m	-0.351	0.727	-0.878	0.385	-0.773	0.443
HTP*T	1.59	0.116	-0.009	0.993	0.881	0.381
HTP*E	2.586	0.011*	2.373	0.020*	2.503	0.014*
AIC:null	47		-427.1		-468.7	
AIC:full	39.4		-434.1		-490.1	
Langtang National Park						
Intercept	2.154	0.036*	25.268	<0.001***	6.664	<0.001***
Harvest treatment percent (HTP)	1.531	0.128	2.644	0.009**	2.543	0.012*
Transition year (T)	-1.246	0.215	1.374	0.172	-0.909	0.365
Elevation (E) m	-1.435	0.157	0.674	0.501	0.229	0.819
HTP*T	-1.201	0.232	-3.875	<0.001***	-3.504	<0.001***
HTP*E	-1.049	0.296	-2.399	0.018*	-2.317	0.022*
AIC:null	142.3		-782.8		-379	
AIC:full	85.8		-817.6		-431.8	

Square root transformation for vegetative fecundity and logit transformation for regeneration and recruitment percentage were used to control for the skewed distribution. The mixed effect models include a random effect of 'Plot' as a random factor. The symbols, $p < 0.0001$ ***, $p < 0.001$ ** and $p < 0.05$ * represent different degrees of significance.

5.4.2 Population Growth

In both study sites, the annual growth rate was highest in the lower alpine (<4250 m) area. In the control sub-plots, growth rates appeared higher in the second year of harvest as plants experienced mild disturbances during locating and tagging in the first year of harvest treatment. For the third year onwards, the growth rate declined towards unity. Asymptotic growth rates were less than unity ($\lambda < 1$) in all the conditions at control subplots of upper alpine ANCA but in LNP they were less than unity only when sexual reproduction was omitted. Thus, the results showed evidence that viable seed production and seedling establishment had a significant impact on the population dynamics (**Table 11**). However, a narrow variation in λ value: 0.923–0.989 for ANCA and a wider variation: 0.935–1.042 for LNP showed that sexual reproduction had a marked contribution to the growth rates. Apparently, clonal propagation showed a marginally positive impact on growth rates in the ANCA but a less remarkable effect for LNP populations where, instead, sexual regeneration showed a greater contribution.

Table 11: Finite rates of increase (λ) along the experimental harvest-gradient for lower alpine (<4250 m) and upper alpine (>4250 m) transition matrices for 2015–2016 and 2016–2017 in Api-Nampa Conservation Area (ANCA) and one more additional transition matrices 2017–2018 for Langtang National Park in the Nepalese Himalaya

λ value based on fecundity type	Transition	Lower alpine (<4250 m)				Upper alpine (>4250 m)			
		0 % (control)	25%	50%	75%	0 % (control)	25%	50%	75%
Api-Nampa Conservation Area (ANCA), north-west Nepal									
Combined clonal and sexual reproduction	2015–16	1.099	1.076	0.938	0.804	0.978	0.993	0.960	0.959
	2016–17	1.087	1.153	1.031	0.927	0.989	0.997	1.044	0.973
Omitting clonal-reproduction	2015–16	1.018	0.979	0.884	0.696	0.925	0.906	0.750	0.821
	2016–17	1.054	1.071	0.963	0.759	0.961	0.890	0.682	0.556
Omitting sexual reproduction	2015–16	0.929	0.978	0.922	0.804	0.900	0.990	0.960	0.959
	2016–17	0.905	0.916	0.882	0.927	0.923	0.940	1.044	0.973
Langtang National Park (LNP), north-central Nepal									
Combined clonal and sexual reproduction	2015–16	1.080	1.087	1.173	0.977	1.019	1.019	0.914	0.965
	2016–17	1.138	1.407	1.656	1.044	1.055	1.086	1.104	0.906
	2017–18	1.097	1.229	1.382	1.226	1.042	1.118	1.075	0.938
Omitting clonal-reproduction	2015–16	1.063	1.047	0.969	0.835	1.008	0.985	0.835	0.814
	2016–17	1.114	1.210	1.249	0.593	1.042	1.031	0.898	0.433
	2017–18	1.086	1.195	1.225	1.062	1.034	1.066	0.936	0.892
Omitting sexual reproduction	2015–16	0.953	0.988	1.075	0.977	0.954	0.968	0.914	0.965
	2016–17	0.892	1.118	1.380	1.044	0.920	0.880	1.023	0.906
	2017–18	0.924	0.841	1.006	0.917	0.935	0.880	0.919	0.933

For combined sexual and clonal reproduction, growth rates were always higher than equilibrium ($\lambda > 1$) for 25% harvest in the lower alpine area of ANCA and for 50% harvest treatment in LNP. Meanwhile, in the lower alpine area of LNP, growth rates increased up to 50% of the harvest and reached more than unity until the second year of harvest, and the population growth was projected to peak, (>80% in the second year from harvest and growth in the later years gradually reduced) under the prevailing environmental conditions (**Figure 16**). However, growth for >25% harvesting treatment was found to be retrogressive in ANCA and >50% in LNP (**Table 10**). Nevertheless, multiyear observations might be reasonable; particularly the second-year transition of ANCA might not provide a realistic perspective on population viability.

Table 12: Examining the effects of experimental harvest, elevation gradient, and transition year on *Neopicrorhiza scrophulariflora* population growth characteristics in Nepal's Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP) using logistic regression

	Progression		Stasis		Retrogression		Mortality	
	Z	p-value	Z	p-value	Z	p-value	Z	p-value
ANCA								
Intercept	-2.122	0.034*	3.042	0.002**	1.621	0.105	3.156	0.002**
Harvest treatment (HT)	2.033	0.042*	-3.713	<0.001***	-1.120	0.263	0.566	0.571
Transition (TR)	0.790	0.430	0.479	0.632	-1.387	0.165	-0.143	0.886
Elevation (E)	2.582	<0.010**	-2.829	0.005**	0.542	0.588	-0.983	0.326
HT*TR	-4.179	<0.001***	-0.482	0.630	3.311	<0.001***	2.216	0.027*
HT *E	-0.892	0.372	3.946	<0.001***	1.063	0.288	-2.129	0.033*
LNP								
(Intercept)	-0.341	0.733	3.761	<0.001***	0.497	0.619	0.463	0.643
Harvest treatment (HT)	-1.697	0.090	2.802	0.005**	0.917	0.359	-1.044	0.296
Transition (TR)	2.625	0.009**	-4.007	<0.001***	4.256	<0.001***	-2.625	0.009**
Elevation (E)	1.484	0.138	-3.302	<0.001***	1.208	0.227	2.607	0.009**
HT*TR	-8.568	<0.001***	2.222	0.026*	2.748	0.006**	6.766	<0.001***
HT*E	2.489	0.013*	-2.337	0.019*	-0.793	0.428	-0.964	0.335

Other p-values are non-significant ($p > 0.05$), and the likelihood of significance is ** $p < 0.001$; * $p < 0.05$.

In the upper alpine populations of both study sites, growth rates were reduced to less than unity ($\lambda < 1$) in all harvest treatments and in all transitions when sexual reproduction was omitted (**Table 11, Figure 16**). Apparently, the plants had a poor sprouting rate and clonal reproduction contributed even less significant to the growth than observed for the lower alpine populations. In addition, plant mortality increased with increasing elevation (**Table 12**). However, combining sexual and clonal reproduction, the upper alpine populations of ANCA were sensitive to harvest, and the 25% harvesting treatment reduced the number of ramets per annum by at least 2-7%

(Figure 16). Regardless of this, upper alpine populations in LNP were more resilient to harvest, and harvesting treatments up to 50% showed almost balanced positive effects, whereas a harvesting treatment of 75% led to a 5-7% reduction in the number of ramets per annum (Figure 16).

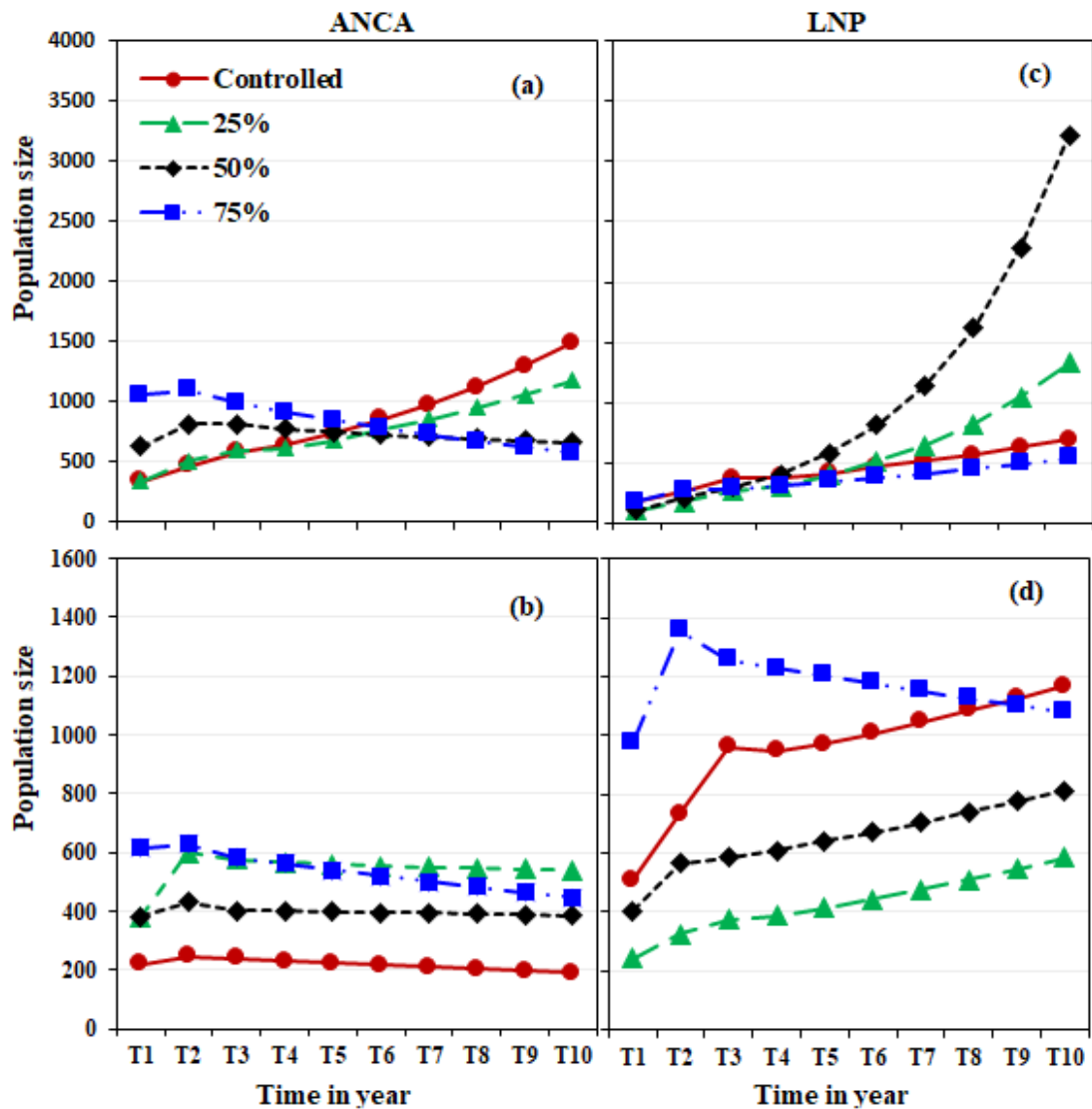


Figure 16: Growth rate projection of population size for next 10 years of *Neopicrorhiza scrophulariiflora* in relation to harvesting treatments in lower alpine and upper alpine areas of Nepal's Api-Nampa Conservation Area (ANCA: a and b) and Langtang National Park (LNP: c and d). The values for lower alpine area of Langtang National Park is numerically divided by three for the graphical adjustment

5.4.3 Stage Class Distribution and Harvest Impact

The proportion of flowering ramets (Rep) was low in almost all studied populations. This was followed by the sprouting category (SP, seedling), and the stable stage

distribution was mostly higher for these classes than for other life-history classes (**Table 13**). Besides, in the majority of cases when the level of harvesting treatment increased, higher values were recorded for the adult vegetative (AV) and juvenile (JV) classes (observed stage class distribution). In the combined data set, the ramets showed a higher probability of progression from smaller to larger-sized ramets in ANCA ($p < 0.050$) by reducing morphological stasis ($p < 0.001$, **Table 12**). The plant response to harvest treatment with respect to sprouting was negative in ANCA and positive in LNP (**Tables 12 and 13**). However, generally, ramets progressed into larger-sized classes when harvest treatments were applied to our experimental subplots (**Table 12**). On average under controlled harvesting treatments, the largest proportion of plants was found in the AV class, which included 57% of the plants in the lower and 49% in the upper alpine population in ANCA and 70% in the lower and 69% in the upper alpine population in LNP.

In control subplots in ANCA, each year on average 8% (range: 7-10%) and 6% (4-7%) of ramets in lower and upper alpine populations undergo sexual reproduction, respectively. In the same situation in LNP, only 6% (5-7%) and 7% (2-8%) of ramets produced flowers in the lower and upper alpine populations. Until the end of 2017, the 25% harvest treatment did not affect sexual reproduction but instead supported it in the lower alpine population of ANCA. However, the effect was the opposite and stronger for harvest treatments exceeding 25% and for populations located in the upper alpine area (elevation >4250 m, **Table 13**).

Remarkably, up to the second transition (2017-18) in LNP, we recorded 11% and 7% flowering ramets in the lower alpine and 7% and 3% in the upper alpine areas for the 25% and 50% harvest treatments, respectively. The ramet mortality rate was critical to the stability of the population as indicated by the value of the Keyfitz index, which was higher in ANCA compared to the LNP (**Table 13**). The Keyfitz index value mostly increased for higher levels of harvesting ($>50\%$), particularly at the upper alpine populations. Nevertheless, the index value was mostly reduced by almost half (in ANCA) and three times (in LNP) later in the 2- and 3-transition for the lower level of harvesting treatment ($\leq 50\%$).

Table 13: Stable stage (Sta.), observed stage (Obs.) distributions, and Keyfitz index of dissimilarity (Δ) of *Neopicrorhiza scrophulariiflora* for different harvesting treatments at lower and upper alpine populations censused between 2015 and 2017 in Api-Nampa Conservation Area and, 2015 and 2018 in Langtang National Park, Nepal

Site and year	Stage class	Lower alpine (<4200 m)								Upper alpine (>4200 m)							
		0 (control)		25%		50%		75%		0 (control)		25%		50%		75%	
		Obs.	Sta.	Obs.	Sta.	Obs.	Sta.	Obs.	Sta.	Obs.	Sta.	Obs.	Sta.	Obs.	Sta.	Obs.	Sta.
ANCA: 2015– 2016	SP	0.00	0.34	0.01	0.34	0.00	0.15	0.00	0.25	0.00	0.22	0.01	0.24	0.01	0.33	0.01	0.30
	JV	0.33	0.25	0.35	0.24	0.33	0.25	0.46	0.47	0.48	0.24	0.45	0.25	0.32	0.36	0.33	0.34
	AV	0.51	0.21	0.47	0.21	0.53	0.29	0.21	0.29	0.44	0.26	0.49	0.26	0.42	0.31	0.23	0.36
	Rep	0.10	0.20	0.06	0.21	0.01	0.31	0.00	0.00	0.04	0.27	0.01	0.26	0.00	0.00	0.00	0.00
	Δ	2.32		5.47		6.66		16.57		1.83		2.76		12.89		21.54	
ANCA: 2016– 2017	SP	0.00	0.35	0.01	0.36	0.00	0.28	0.00	0.28	0.00	0.23	0.01	0.25	0.01	0.36	0.01	0.32
	JV	0.27	0.25	0.28	0.25	0.21	0.25	0.20	0.33	0.35	0.25	0.35	0.25	0.31	0.33	0.25	0.34
	AV	0.62	0.21	0.56	0.21	0.63	0.24	0.64	0.39	0.54	0.26	0.47	0.25	0.49	0.31	0.49	0.35
	Rep	0.07	0.20	0.10	0.19	0.05	0.23	0.00	0.00	0.08	0.26	0.04	0.25	0.00	0.00	0.00	0.00
	Δ	1.90		3.07		5.60		7.80		1.45		6.65		9.73		12.70	
Overall ANCA	SP	0.00	0.33	0.01	0.35	0.00	0.23	0.00	0.24	0.00	0.23	0.01	0.24	0.01	0.35	0.01	0.31
	JV	0.30	0.24	0.31	0.25	0.27	0.25	0.35	0.37	0.42	0.25	0.40	0.25	0.31	0.34	0.30	0.34
	AV	0.57	0.23	0.52	0.21	0.58	0.26	0.40	0.39	0.49	0.26	0.48	0.25	0.46	0.31	0.35	0.35
	Rep	0.09	0.20	0.08	0.19	0.03	0.26	0.00	0.00	0.06	0.26	0.02	0.25	0.00	0.00	0.00	0.00
	Δ	2.10		4.19		6.12		12.68		1.63		4.84		11.31		17.55	
LNP: 2015– 2016	SP	0.01	0.42	0.01	0.44	0.01	0.39	0.01	0.36	0.00	0.31	0.01	0.30	0.00	0.32	0.01	0.39
	JV	0.24	0.22	0.22	0.22	0.31	0.24	0.16	0.34	0.28	0.24	0.24	0.24	0.19	0.37	0.19	0.33
	AV	0.61	0.19	0.56	0.18	0.46	0.19	0.48	0.30	0.64	0.23	0.61	0.23	0.66	0.31	0.38	0.28
	Rep	0.07	0.18	0.09	0.17	0.02	0.18	0.00	0.00	0.06	0.23	0.04	0.23	0.00	0.00	0.00	0.00
	Δ	3.65		6.16		9.74		17.50		0.69		5.21		7.39		21.20	
LNP: 2016– 2017	SP	0.00	0.40	0.01	0.46	0.02	0.47	0.01	0.37	0.00	0.34	0.01	0.32	0.01	0.32	0.00	0.30
	JV	0.15	0.23	0.12	0.22	0.21	0.24	0.22	0.33	0.39	0.24	0.27	0.25	0.27	0.25	0.27	0.35
	AV	0.72	0.20	0.68	0.19	0.54	0.17	0.56	0.31	0.04	0.21	0.58	0.22	0.60	0.23	0.59	0.36
	Rep	0.07	0.18	0.08	0.13	0.08	0.12	0.00	0.00	0.02	0.21	0.05	0.21	0.02	0.21	0.00	0.00
	Δ	2.74		4.75		7.42		10.80		27.37		4.53		5.07		6.77	
LNP: 2017– 2018	SP	0.00	0.41	0.01	0.39	0.01	0.41	0.01	0.34	0.00	0.34	0.01	0.35	0.00	0.30	0.00	0.21
	JV	0.13	0.22	0.19	0.26	0.21	0.26	0.11	0.23	0.07	0.24	0.15	0.25	0.25	0.26	0.18	0.24
	AV	0.75	0.19	0.61	0.19	0.56	0.19	0.58	0.24	0.06	0.21	0.67	0.21	0.61	0.23	0.66	0.27
	Rep	0.05	0.18	0.11	0.16	0.08	0.14	0.08	0.19	0.87	0.21	0.07	0.19	0.04	0.22	0.02	0.28
	Δ	3.25		3.82		7.01		10.87		0.00		4.63		5.09		6.85	
Overall LNP	SP	0.00	0.41	0.01	0.43	0.02	0.43	0.01	0.31	0.00	0.33	0.01	0.33	0.01	0.29	0.00	0.24
	JV	0.17	0.22	0.18	0.23	0.23	0.25	0.16	0.25	0.19	0.24	0.22	0.25	0.24	0.25	0.21	0.25
	AV	0.70	0.19	0.62	0.18	0.53	0.19	0.54	0.23	0.69	0.22	0.62	0.22	0.62	0.23	0.55	0.25
	Rep	0.06	0.18	0.10	0.15	0.07	0.14	0.03	0.21	0.07	0.21	0.05	0.21	0.02	0.23	0.01	0.26
	Δ	3.20		4.77		7.81		12.95		2.19		4.77		5.70		11.05	

5.4.4 Sensitivities, Elasticity, and Reproductive Value

There was some variation in both sensitivity and elasticity structures among the harvesting treatments and along the elevation gradient in both study sites (**Table B.14 and B.15**). In the control subplots of LNP, we have very few observations of transition from Sprouter (SP) to the Adult vegetative class (AV), which showed the greatest value of sensitivity (**Table B.14**). However, in ANCA, the pattern was not as uniform as in

the LNP (**Table B.14**). There were greatest sensitivity values for the transition from SP to Juvenile ramet (JV) in the first transition (2015-2016) and to AV in the second transition (2016-2017). The Adult ramets also included a relatively high number of individuals that had sprouted vegetatively within a year.

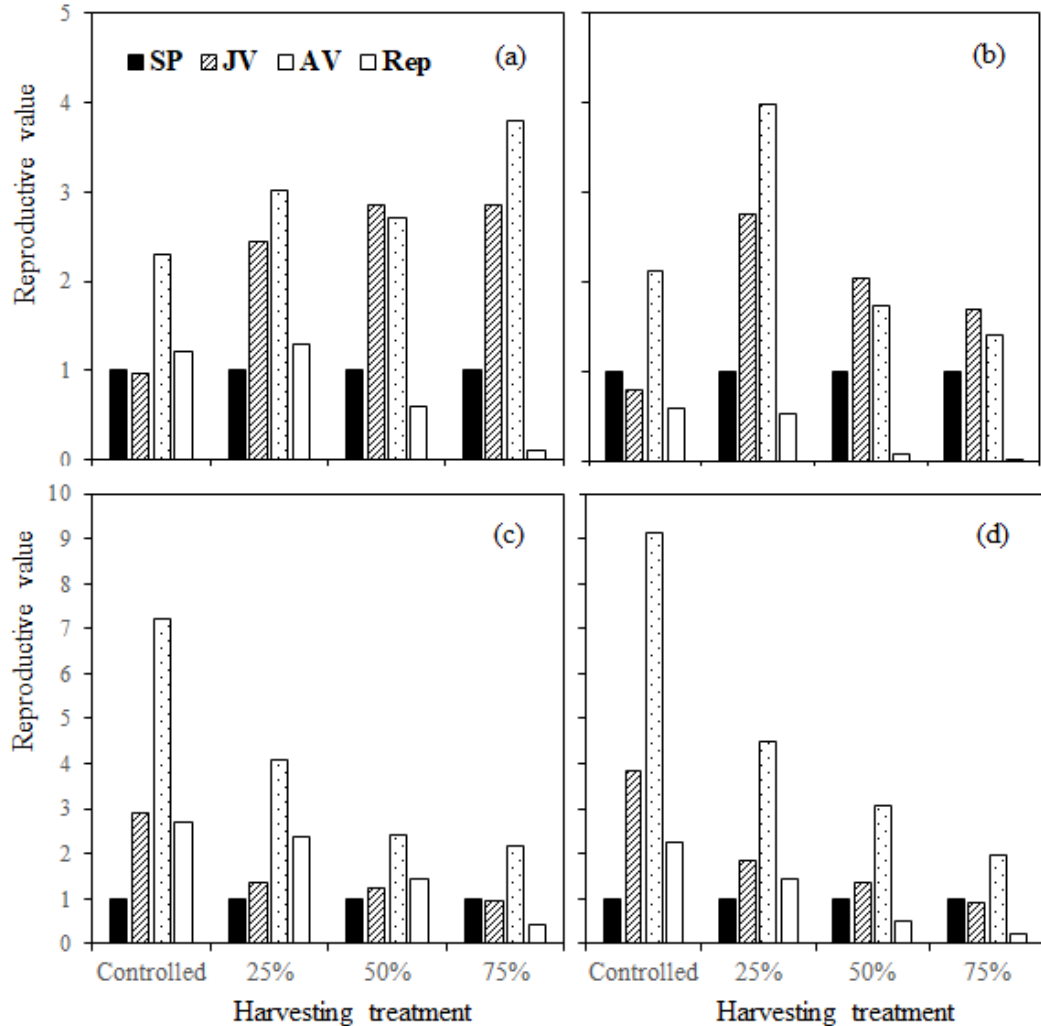


Figure 17: Life history stage-wise reproductive value along the harvesting treatments in lower and upper alpine populations of *Neopicrorhiza scrophulariiflora* censused between 2015 and 2017 in Api-Nampa Conservation Area (a and b) and, 2015 and 2018 in Langtang National Park (c and d), Nepal

The transition matrix elements with the greatest proportional contribution to the finite rate of increase in the control subplots of the lower alpine area in the ANCA were the stasis of juvenile ramets in the first transition and adult ramets in the second transition, and such contribution was just reversed in the control subplots for the upper alpine population (**Table B.15**). For higher levels of harvesting treatments, mixed patterns were observed with regard to stasis of either juvenile ramets or adult ramets. While in the LNP, for the control subplots, the elasticity value of adult

vegetative (AV) ramet for all three transitions (2015-2018) years provided the greatest scale of proportional change to the population growth rates (**Table B.15**). However, the relative contribution of AV for the population growth rate at a higher level of harvest treatments (>control) was not consistent as in ANCA.

For the lower alpine populations at both sites, higher sexual reproduction was coupled with higher survival in adult stages and lower ramet mortality (**Table 12; Figure 17**). The contribution of reproduction of the middle static stage (JV and AV) of *Neopicrorhiza* was generally higher in both study areas compared to sprouting and reproductive stages, due to the lower mortalities of those stages (**Figure 17**). In ANCA, AV and JV had almost identical reproductive values in most of the harvesting treatments. In LNP, the AV life stage showed the greatest reproductive value followed by JV and the values were even higher for the control plots and for the higher elevation populations (**Figure 17**). Reproductive values and harvesting treatments were positively associated in the lower alpine area of ANCA but in other cases, the association was negative or unclear.

5.5 Discussion

5.5.1 Harvest Impacts on Fecundity, Regeneration, and Recruitment

The impact of harvesting was not always deleterious to population parameters such as fecundity, regeneration, and recruitment. To a certain degree, harvesting induces adaptive changes in the harvested populations (Poos *et al.*, 2011). As evidenced in our study, sexual fecundity was sensitive to the harvest, and a higher rate of removal of ramets left the majority of the population in the vegetative stage, which is analogous to other studies where a higher level of experimental harvest reduced pollination, fruit production, and production of viable seeds (Baldauf *et al.*, 2015; Chen *et al.*, 2015). However, a higher rate of sprouting in lower elevation populations stabilized the population and, in some cases, the overall growth rate increased, as in the lower alpine area of LNP. Previous studies (Endress *et al.*, 2004; Ghimire *et al.*, 2008a) have stated that clonal propagation in clonal plants increases with elevation, whereas sexual reproduction decreases, as also observed in this study (**Table 11**), and that plants tend to allocate more biomass for vegetative traits compared to reproductive traits. Thus, plants exhibit a greater potential for forming bud banks and higher tolerance against mild disturbance (e.g. in relation to rotational and managed harvest) and consequently,

re-sprouting might exceed that of plant mortality. Under such conditions, harvesting may have a positive impact on regeneration. Also in the meantime, more substrates may be available for the growth of a reduced number of rhizomes, and, this way, new sprouters will experience sufficient space, and resource acquisition might be faster which might speed up colonization.

The negative impact of harvest such as mortality could be defeated by the regeneration developed from the inherent bud bank of the plants. Thus, together a low mortality and a high regeneration potential lead to a high recruitment and growth of the population. Also, a fraction of the resprouting might be related to rhizome fragments that were left unintentionally by the harvesting treatments in the experimental plots, which is similar to the experience reported for another clonal herb, *Ligusticum porteri* (Kindscher *et al.*, 2019). In that specific case, clonal twigs or plantlets had the faster ability to root, greater chances for the establishment, and quicker progression than seedlings developing from seed. By contrast, in the alpine population, all levels of harvesting treatments in ANCA and harvesting treatments exceeding 50% removal of plants in LNP turned out to be detrimental to regeneration, recruitment, and fecundity.

5.5.2 Population Growth Parameters and Vital Stages

In general, *Neopicrorhiza* is progressed to higher stage classes when harvest treatment applied, however, spatial and temporal variation was observed in this study. Also, the harvest treatment exhibited an almost reversed relation with the stasis of the ramet. This evidence indicates that sustainable extraction of *Neopicrorhiza* is possible under given site-specific conditions (**Table 12**). Although under experimental harvesting, plant stasis reduced as elevation increased, the high rate of mortality in those areas indicated that plant response to harvest impact cannot be generalized. As stated in previous studies (Ghimire *et al.*, 2008a; Hautier *et al.*, 2009), compared to low-elevation hospitable habitats where plants get an almost year-round growing season, the upper alpine species are more sensitive, due to the limited number of growing days, low temperature, greater ultra-violet radiation, and inadequate soil nutrients, which make it harder to maintain population gene pools through reproduction and growth; and this is exacerbated by harvest impact. Thus, we suggest that using the same intensity of harvest as in lower alpine areas might be unacceptable, as it may not allow upper alpine populations to maintain viability.

Survival of adult ramets (particularly AV and JV) contributed more to the population's projected growth rate and was found to be a key demographic driver for the population recovery of *Neopicrorhiza* after harvest. In the control condition, population demography was characterized by high adult survival, high values of elasticity, and high reproductive value (**Figure 16**) in those life stages. However, their relative contribution of proportional change to growth rate along the harvest treatments and spatial conditions was not uniform. Thus, a selective harvest strategy for a particular plant stage may not support the sustainable management of the population. This is in agreement with observations made for other traded rhizomatous plants such as *Hydrastis Canadensis* (Van der Voort *et al.*, 2003) and *Ligusticum porteri* (Kindscher *et al.*, 2019). The advancement of the seedling stage to higher classes within a year was found to be a unique characteristic of *Neopicrorhiza* in our study. Nevertheless, over the course of the study, covering three years in ANCA and four years in LNP, no seedling matured to flowering-reproductive status. Studies have shown that the relative role of reproduction declines as the life span increases in polycarpic perennial plants (Klimeová *et al.* 2012; Chen *et al.* 2015; Rokaya *et al.* 2017). Episodic harvests might affect the resource allocation of mature sterile ramets due to the expected trade-off between clonal sprouting and sexual reproduction, and in our study, clonal sprouting of mature sterile ramets was apparently promoted. The threshold age for flowering maturity of such ramets exceeded the duration of this study and therefore remains to be determined in future studies.

The larger lambda value for the second transition (2017) in both sites was high in most cases due to increased vegetative sprouting. Harvesting treatment enhances sprouting and increases colonization opportunities by providing vacant space. Modification of the microhabitat caused by the harvesting treatment could further benefit the recruiters. The impact of a transition year in ANCA populations was less prominent but in LNP increasing transition years reduced the ramet mortality and morphological stasis and induced advancement to higher-class stages. This clearly indicates that sustainable harvest is possible if multi-year rotations are applied.

5.5.3 Optimal Harvest Regimes and Sustainable Extraction

For sustainable harvesting, we propose spatio-temporal harvesting approaches with harvests at least at 4-year intervals, so that plant populations can achieve sufficient

recruitment and adequate viability. The lower level of harvesting ($\leq 50\%$ removal of ramets) in the lower alpine population of ANCA recovered in the second transition year (2017) and one year earlier in the populations at both elevation ranges of LNP (**Table 10**). Based on this evidence, sustainable harvest of ramets could generally be achieved by using harvesting intervals of at least three years for lower alpine populations. However, growth rates in the upper alpine range of ANCA were retrogressive in all transitions studied, even for the control subplots, implying that populations may not be able to sustain any level of harvest. Aside from that, the annual removal of 25% ramets did not affect the growth rate in LNP and could lead to a stable population.

Nevertheless, caution is needed when interpreting the evidence. The harvest treatments we used in experimentation were meant to simulate local harvesters' behavior, and this behavior may not in the practice match what is done in the experimental treatments although the range of intensities should be covered (for example the local harvesting may involve additional trampling and additional excavation of only partly developed new sprouts). The estimated growth rates in these findings are based on deterministic projection, whereas in practice, growth will vary considerably as a consequence of random factors such as weather, precipitation, and the potential occurrence of landslides in the steep terrain. The observations that form the basis for estimating the transition parameters in this study are also uncertain because a limited number of plots covering a limited area are included in this finding.

5.6 Conclusion and Future Research Directions

The study highlights that plant response to harvest varied with elevation and over time. Vegetative growth and sexual reproduction are traded off as a result of harvesting; sexual reproduction declines as harvest intensity rises; and, to some extent, clonal recruitment of the plant partially offsets the negative effects of harvest. Clonal recruitment showed a marginally positive impact on the growth rate in ANCA, but this effect was less remarkable for LNP populations. Instead, in LNP, sexual reproduction widely increased the growth rates. Thus, the hypothesis; 'all levels of harvest may not be detrimental to the plant's fecundity, regeneration, and recruitment; and spatial and temporal response to plant extraction may determine the fate of the population and the optimal harvest limit', is supported by our study. For areas experiencing the high intensity of harvest like in ANCA, up to 25% removal of plants seems to allow

maintaining the pre-harvest population if operating with 2-year harvest intervals. By contrast, in the strictly protected area in LNP, the removal of up to 50% of the plants did not affect the growth rate of the population, instead, growth rates were higher than in the control plots, most clearly in the lower alpine population. Hence, we propose at least 4-year harvesting intervals for the sustainable extraction of *Neopicrorhiza* population so that sufficient fecundity and recruitment can be achieved, thus maintaining population viability.

The study does not enable us to assess how long time it would take for the upper alpine population in ANCA to recover to the pre-harvest condition after harvesting treatments $\geq 50\%$. It revealed that recovery was observed in LNP for these treatments, but the point is that in ANCA no tendency of recovery was observed for these treatments. Also, the study does not enable us to say what would happen if repeated harvest beyond our protocol was carried out in the experimental harvested subplots. As harvests happen regularly outside of the experiments—possibly annually, multiple times in a single year, and at intervals of a few years—anytime a harvester passes by and decides that a resource of sufficient size and quantity is there. Additionally, due to the short study period, we were unable to discuss the different climatic factors, such as the length of the growing season, precipitation, solar radiation, and the resulting changes in edaphic factors experienced by *Neopicrorhiza* populations in the Himalayas. For example, we were unable to discuss what would happen if harvesting took place in a year of drought as opposed to a year with abundant rainfall. So, these issues could be the subject of future research.

CHAPTER 6

6.1 SUMMARY AND CONCLUSIONS

6.1.1 Synthesis of the Findings

In the Himalayas, a considerable quantity of MAPs are collected from the wild for local medical needs and to generate cash income. Harvesting and increasing trade of high-altitude MAPs like *Neopicrorhiza scrophulariiflora* are considered integral means of improving rural economies and providing an important source of income to rural households. Nonetheless, the commercial harvest for regional and international trade has created extreme pressure on plant populations and their natural habitats. As air-dried crude rhizomes are traded along well-established marketing chains, prices are 600-1000 NRs (5-9 USD) kg⁻¹ at the harvester level and increase to 2500 NRs at the transboundary level. Nepal is the largest global supplier of wild-harvested air-dried rhizomes to the international markets. However, the current lucrative markets and increasing prices for the products cause serious concern for the long-run survival of populations in threatened habitats. Thus, the dissertation was written in an effort to solve this issue; the findings may aid in the development of measures for the sustainable harvest that are crucial for the socioeconomic well-being of rural populations in the Nepalese Himalayas as well as for the conservation and management of the species. **Table 14** provides a general summary of the findings, novelty, and limitations of the dissertation.

Chapter one is introductory and explains the framework of this research. In chapter two, the study was carried out using distribution data based on specimens deposited in national and international herbaria, and suitable and unsuitable habitats were identified through Maximum entropy modeling. The trade data were accumulated from unpublished work provided by government agencies of Nepal. The potential distribution area for *Neopicrorhiza scrophulariiflora* is 11617 km², particularly in the highland areas of Nepal, and comprises about 8% area of the country, predominantly in the eastern region. Highly suitable areas (386 km², about 0.3%) tended to be located in the narrow altitudinal range: 4000–4400 m. The plant favors mild temperatures and adequate precipitation in the peak growing months (June-August). The habitat suitability decreases from the eastern towards central and western regions of Nepal due

to the lower precipitation and higher temperature, however, the harvest intensity is heavy in the western regions.

Table 14: Synopsis of the dissertation outlining major findings, novelties, and limitations

Chapters	Chapter: 2	Chapter: 3	Chapter: 4	Chapter: 5
Major findings	Local populations are dwindling as a result of overexploitation and heavy trade in regions with climatically unfavorable habitats for the species.	As per the continuing local harvesting system, low harvest intensity is resilient to the populations and had no adverse impact on populations	Harvest recovery was high in lower elevation populations. Harvest recovery was far slower at higher elevations (>4250 m), and harvest intensively (>50% extraction) recovered very slowly.	Plants removal up to 25% at the upper alpine and 50% at the lower alpine populations at the interval of 4 years is resilient to the population growth and permitting sustainable harvesting.
Novelty	The determination of suitable habitats; interrelationship among availability of such habitats, the pattern of resource extraction and the trade history are explored together which, to our knowledge, has never been done before.	Relationships of environmental parameters including de facto harvest with key biological parameters of plant are essential elements in sustainable management.	Postharvest recovery rate and factors affecting recovery along the elevation gradient in the Nepalese Himalaya are the new scientific discourse.	Introduction of clonal and sexual fecundities to estimate the population growth rate providing novel methodological insight. Population dynamics along the harvest intensity across the continuum of elevation and conservation gradient (protected vs. open area) help to estimate the optimal harvest limit which is a new paradigm from the Himalaya.
Limitation	Future scenarios are not analyzed that could be relevant due to climate change, presumably leading to increased temperature, changing precipitation patterns and changes in the frequency of drought. We could not covered information about the growth and reproductive capacity of the plants in different parts of the distribution range.	The effects of environmental factors is analyzed only for plant density and biomass, other aspects like plant phenology, germination, and establishment need to be addressed in relation to a continuum of environmental conditions.	We could not make equal post-harvest monitoring, and covered only 2 transition years in ANCA and 3 in LNP.	The analysis is only based on deterministic growth rates and could not include life-table response experiments.

Available official records indicate that a total of 372 tons (around 31 tons/annum) of dry rhizomes were exported (which amounts to around 600, 000 USD) to international markets in the 12 fiscal years, which is far lower than expected, presumably due to poor official records and illegal harvest as explained in detail in

chapter two. It clearly indicates that suitable habitats are isolated and infrequent in the Nepalese Himalayas. Heavy exploitation occurs mainly from areas of low suitability, particularly in the western mountains, and price and export rates increase year after year. This shows that existing resource exploitation is not sustainable. We reported abundant highly suitable habitats in eastern and central Nepal, which can be given priority when developing agro-technological practices, including domestication, to help align practice with government policies.

Chapters three, four, and five are developed based on empirical field evidence. In chapter three, data was collected to examine the effect of harvest (controlling for environmental factors at the respective habitats) on plant density and biomass from sites with contrasting conservation and harvesting regimes in central and western Nepal. Both the Langtang National Park (LNP) in central Nepal and the Api-Nampa Conservation Area (ANCA) in western Nepal were chosen as case study locations because they offer protection and commercial open access, respectively. An equal volume of rhizomes is harvested for traditional health care in both ANCA and LNP. While in the ANCA, such effects were unfavorable for plants at all stages, in the LNP, the impact of recent harvest on plant density was beneficial for vegetative and negative for reproductive individuals. In the intact condition, shrubland meadows had high densities of plants, but higher densities were recorded in rocky habitats in the ANCA populations due to ongoing due to repeated harvests.

Findings in chapters four and five emphasize the postharvest recovery rate; population growth and dynamics along the gradient of harvesting treatments. Moreover, plants' response to harvest with respect to fecundity, regeneration, recruitment, survival, and mortality are also discussed. We explored the population viability and fate of populations to find optimal harvest limits. The lower level of harvesting was found to enhance the bud bank potential and vegetative sprouting and apparently increased harvesting opportunities. Repeated recovery analysis shows that density and reproductive outputs varied significantly among the harvesting treatments and co-varied with the pre-harvest plant number. In lower alpine populations at the LNP, both density and reproductive output were restored within three years after experimental harvesting treatments with intensities of up to 50% removal of plants. Generally, based on demographic analysis (growth rate: λ value), 25% harvesting treatments at intervals of two years is stable for almost all studied populations. Specifically, for the lower

alpine population in the LNP, the growth rate was stable up to 50% for the same transition period, and it was noticeably higher than in the unharvested (control) plots. Plots harvested >50% extraction became exhausted which affected both harvest recovery and population growth rates in both elevation levels of both sites. Growth was negative even in the control plots for populations located higher than 4250 m, particularly in ANCA, and on this account, restoration to harvesting treatments >25% is only achievable to a limited extent in those areas.

For sustainable harvesting and management, we propose spatiotemporal harvesting approaches adapted to prevailing climatic conditions. In general, except in populations where exploitation occurs repeatedly and the climate is hostile to regeneration like in the upper alpine population of ANCA, we suggest harvesting at intervals of at least 4 years, so that plants can achieve sufficient recruitment and adequate viability. Caution is needed, where postharvest recovery appears slower, for example, the negative effect of the harvest was less reparable in the upper alpine populations at elevations greater than 4250 m in both sites. The study came to the conclusion that harvesting implies a trade-off between vegetative growth and sexual reproduction, with sexual reproduction declining as harvest intensity rises. To some extent, the clonal resprouting and fugitive growth of the plant counteract the negative impact of harvest. Moreover, clonal propagation may be varied with precipitation, soil type, solar radiation, and temperature along the geographical gradient. Thus, it is necessary to adjust the harvest regime to local conditions. Spatiotemporal population management and harvest regulation are needed for long-term conservation and maintenance of the resource and for achieving a sustainable supply.

In a nutshell, the dissertation addressed: (i) how exploited MAP species—such as *Neopicrorhiza scrophulariiflora* shape their distribution and availability of resources with ecological factors; (ii) how natural regeneration and restoration following harvest are determined; (iii) how the demographic parameters of exploited plant populations respond to changing environments; and (iv) how the current trade channel affects the distribution and availability of plant resources and what regulation scheme is needed, thereby supporting sustainable harvesting practices that enable local livelihoods. The findings aid in filling up the information gap for trade-exploited Himalayan MAPs and can be replicated to research frontiers beyond the Himalayan region.

6.2 RECOMMENDATIONS

6.2.1 Recommendations for the Sustainable Harvesting and Management

(a.) Plant populations may exist in eastern and central Nepal for potential harvest:

The *Neopicrorhiza* distribution map we developed in this study (Chapter 2) provides baseline information for setting up management policies for the sustainable conservation of threatened habitats, particularly in western Nepal. At the same time, the map indicates that a considerable potential for harvesting plant populations exists in the eastern and central mountains of the Nepalese Himalayas. Based on the assessment of these stocks, the annual sustainable harvest quota could be determined and disseminated at the local level. The high mountains of eastern and central Nepal showed a high concentration of highly suitable habitats, which declined towards the western region. Such habitats could be employed for experimental cultivation of the species so that these habitats could help fill the gap when lowering the overharvesting pressure in western Nepal. We propose that even in the western region, some areas, such as the Dolpa and Mugu districts, have relatively significant winter precipitation, and that these areas could be supported for experimental wild cultivation in order to combat commercial overexploitation.

(b.) Harvest can be permitted up to 25% extraction for upper alpine and 50% for lower alpine populations:

Based on the empirical evidence, at a lower elevation, low intensity of harvest enhances the growth rates of the populations compared to the control (unharvested) in the harvest experiments. Based on density restoration and growth rate, we recommend up to 25% extraction for upper alpine and up to 50% extraction for lower alpine populations at intervals of four years. However, >50% harvesting reduces the population growth and did not recover to the preharvest level in our study duration. Caution should be taken when implementing harvesting programs, particularly for the populations at elevations greater than 4250 m in the area opened for commercial harvest.

(c.) Spatiotemporal harvesting regulation practices needed for sustainability:

As stated in chapter five, the transient dynamics of plant life-stage classes and their growth rates differ greatly across the lower and upper alpine areas, indicating that different harvesting strategies and management actions should be taken to support populations

in the two areas. Plants respond to harvest and the sensitivity of populations to harvesting differs along the elevation gradient. Hence, we strongly recommend introducing spatiotemporal harvesting regulation practices for sustainable management, so that plants can have sufficient recruitment and adequate viability.

(d.) At least 20–25% fruiting ramets should be kept intact in each harvest event and some shrubland pockets must be preserved: A higher level of harvest increases plant mortality, fruit abortion, and reduces reproduction rates. Due to guerrilla strategy and fugitive establishment, selective harvesting applied to particular plant stages is less practicable for a herbaceous species like *Neopicrorhiza*, at least 20–25% of fruiting ramets should be left intact in each harvest event to maintain the genetic diversity of the population. We strongly recommend a rotational harvest with a minimum of three to four years (harvesting rotation can be shorter for lower-elevation populations) between harvests so that plants get a minimum amount of time for maturation. Management authorities should monitor the situation and more precisely, if 25% of ramets have not flowered, harvesting should not be permitted in those populations. Plant density was recorded higher in shrubland meadows, compared to grassland and rocky areas (Chapter 3). Therefore, the preservation and management of shrubby meadows would support the conservation of the *Neopicrorhiza* gene pool.

(e.) Declaration of harvesting months and decision on collection: The present findings could be used as the baseline for policy reformation, as *Neopicrorhiza* is included in a list of species protected by the government of Nepal. The empirical evidence provided in this dissertation thereby supports government decisions on where and when to allow or ban the collection, harvest, and transport of the plant. Generally, the plant starts sprouting in early June, blossoms in August and the fruit matures and disperses in September but after the late-November plants start senescing and hibernate under snow cover. This implies that post-maturity months, such as late September and October, should be declared as harvesting months. However, precautions are needed when issuing harvest permits in the upper alpine populations where short growing periods and low temperatures shrink the plant maturity days. So, the number of harvesting days could be lesser and delayed slightly as elevation increases. Again, caution is needed because if the harvesting period is too late, harvesting is no longer practically possible because of frost.

(f.) Integration of scientific and local knowledge, local empowerment, and value link development is needed: Our findings on experimental harvesting (Chapters 4 and 5) should be integrated into the traditional local knowledge for harmonized management. Local traditional harvesters were aware of the phenology of plants like flowerings, fruitings, seed settings, and dispersal. The results showed limited negative effects on plants by local harvesting for traditional uses. However, at present, unskilled people participate in the harvest in the sites open for commercial collection and reap as much as they can without taking care of traditional norms and scientific basis. As per the present federal government structure of Nepal, the local government holds rights over natural resources like MAPs and can take immediate actions to promote good stewardship practices and can develop harvesting strategies for sustainable management. Accompanied by scientific evidence, local governments should receive support from the local people for the development of a strategic management plan. As per our field experiences (Chapter 3), particularly in the ANCA, harvesters should be trained to provide pre-harvest precautions and post-harvest management. The authority should register harvesters, and authority could issue species-specific permissions depending on the stock available in the respective areas (site-based) to avoid overexploitation, they should be committed before harvesting and their activities should be monitored during harvests. Aside from this, given their efforts and the cost involved, locals were compelled to sell *Neopicrorhiza* rhizomes at too low prices. Therefore, local governments could provide training on post-harvest processing to help increase the value of the products, e.g. through segregation, proper drying, packaging, product branding, and certification that may support higher economic returns.

6.2.1 Limitations and Recommendations for the Future Research

(a.) Collection of field-based evidences are made only in central and western Nepal: In this study, the field-based evidence (population dynamics against *in situ* harvesting treatments) was collected only from two districts (Raswa and Darchula) of central and western Nepal, very limited information is available from eastern Nepal until this date. Nevertheless, based on GIS modelling at present climatic conditions, potentially suitable habitats were concentrated in eastern and central high mountains, and habitats were less suitable and mostly unsuitable towards western Nepal where the

majority of harvesting, trade, and transportation are occurring. As a result, it appears that current *Neopicrorhiza* resource utilization is unsustainable. It is necessary to undertake a thorough empirical investigation on the harvest response of plant populations prior to receiving a harvest permit in order to determine the best annual extraction in at least 20 districts where *Neopicrorhiza* occurs.

(b.) Number of transition years could be increased: We were successfully able to cover three annual transitions (4 years' observation) in LNP to collect data on population growth rates. The number of transition years had to be increased to provide a more ironic decision than the one presented in this research, even though baseline information is gathered. Nevertheless, due to geographical remoteness and limited transport facility, this study squeezed with covering only two transitions (3 years' observation) in ANCA in a given budget and time frame. Therefore, we recommend to consider longer-term monitoring that would clarify inter-annual variation and the connections between the local climate and phenological patterns like flowering and fruit setting and development.

(c.) Alternatives of wild-harvest (cultivation and domestication) is needed: The growing demand for MAPs not only increased the pressure of harvest from the wild but also increase pressure to find alternatives such as cultivation and domestication. The link between existing agricultural cultivation practices, cultural practices, and livelihood practices should be established to convert wild to harvest in local areas. However, caution is needed, as modification of morphological characteristics and loss of gene pool may occur when a plant is cultivated in the socially managed agricultural system due to artificialization of the biophysical environment. Nevertheless, the conservation of species populations in *in situ* wildlands is the best solution for gene pool protection. Commercial production through micro-propagation by maintaining a gene pool could be one of the best research problems to be solved for future endeavors.

(d.) Soil parameters and edaphic changes need to be included: The present study was focused mainly on field-based evidence. Soil parameters including micro- and macro-element have a strong support on the plant growth and post-harvest maintenance. In this dissertation, the role of soil-pH was clearly mentioned (Chapter 3) but the soil structure, water-holding capacity, and nutrient elements are not covered. Thus, future studies should plan in this direction to cover all edaphic factors in the

support of the development of a comprehensive management plan for production and supply.

(e.) Effect of harvest on genetic constitution of the plant needs to accommodate for the future studies: Overall alteration of genetic composition after harvest and their impacts on evolutionary tendencies and biosynthesis of secondary metabolites could be a novel issue to the *Neopicrorhiza* for future research. There may be changes in life history and morphology attributed to intensive exploitation. Also, we are unable to state the best time for the harvest when secondary metabolites are high and the consequential influence of environmental factors on the final products of those chemical compositions. The resultant undesirable evolutionary impacts due to intensive harvest could be a novel issue for the future researchers.

(f.) Impact of recent climate change on wild-harvest and physiological modification need to accommodate for studies: The optimal growing conditions may be influenced by minute changes in the temperatures, precipitations, and availability of nutrients in the soil. The possible modifications on secondary metabolite production leading to reducing drug efficacy are another challenge for plant populations in those places. Due to growing global climate changes, climatological perturbation and their consequential adverse impacts on demography are inevitable for the MAPs like *Neopicrorhiza* growing in the mountain extremes of the Himalayas. Furthermore, the impact that recent climatic changes have had on plant phenology, seed maturation, intact-seed production, germination, and population viability over the long term. Thus, multidisciplinary approaches including physiological, phytochemical, biochemical, and molecular processes and their links to drug development and their responses to changing global scenarios should be advocated through future researches.

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APPENDICES

A. Kutki: Etymology, History of Taxonomic Details, and Key Identifying Characters

Background

Picrorhiza is a commercially threatened Himalayan taxon and encompasses four species (*P. kurrooa* Royle ex Benth., *P. tungnathii* Pusalkar, *P. scrophulariiflora* Pennell, and *P. minima* R.R. Mill) distributed from eastern Himalaya to western Himalaya. The whole genera including all species are popularly recognized by the famous name 'Kutki' besides other local vernacular names (Katuke, Katuk, Karvi, Kadvi). The genus *Picrorhiza* was established by J.F. Royal (1835 cited in Pusalkar, 2014) and later described by G. Bentham in 1885 by the *Type* taxon *P. kurrooa* (Rawat *et al.* 2013). Epithetically, *Picrorhiza* consisting two words: 'Picor' and 'rhiza' which means bitter and root respectively in Greek, and in the case of *P. kurrooa*, the specific epithet is derived from Panjabi 'Karu' which also gives the same meaning of bitterness (Smit, 2000; Rawat *et al.*, 2013; Pusalkar, 2014). The name 'Kurrooa' is usually mentioned as an ingredient by pharmaceutical industries. The nature of this bitterness due to the presence of secondary metabolites terpenoids, glycosides (iridoid glycosides, phenolic glycosides, and phenylethanoid glycosides), cucurbitacin, kutkisterol, steroids in underground part has characterized the development of medicinal properties.

The rhizomes of all the species *Picrorhiza* are commercially traded in amalgamated form and externally rhizomes of all species are not distinguishable. Regarding the biological activity of the rhizome of all the species, most of the works of literature are based on the chemical constituents determined in *P. kurrooa* (Smit, 2000). Besides the kutki allies, in some of the cases, the roots of *Gentiana kurroo* (Gentianaceae) and *Lagotis cashmiriana* (Selaginaceae) grown by similar elevation and having similar root morphology are also mixed up for commercial purposes (Bhattacharjee *et al.*, 2013).

Hooker (1885) first described and treated a single genus *Picrorhiza* having dimorphic flowers by the observation of species collected from the western and eastern Himalayas. Later, Pennel (1943) clarified the misconception of Hooker and established the two species individually in which *P. kurrooa*, a western Himalayan species possess actinomorphic flower with far longer stamens compared to corolla length and *P.*

scrophulariiflora, eastern Himalayan species characterized by bilabiate flower having slightly shorter stamens form. Again, Hong (1984) split this monotypic genus *P. scrophulariiflora* into two generic forms: *Picrorhiza* and *Neopicrorhiza* based on pollen morphology whereas the former is treated by *Scrofella* type of pollen grains and later by *Paederota* type of pollen grains. Based on his finding, the species collected from Bhutan, China (including autonomous region Tibet), Nepal, and Sikkim Himalayas are usually treated as *N. scrophulariiflora*. The monotypic generic form of *Neopicrorhiza* by Hong (1984) did not remain any longer than Mill (2000) and he described additional species *N. minima*, endemic species to Bhutan based on the unique shape of anther. The anther is broader than its length and thecae tips expand outwards from the filament in comparison to elongated anthers with thecae parallel to the filament in the case of *N. scrophulariiflora*.

The specific name of the *P. scrophulariiflora* is also varies in literature *P. scrophulariaeflora* and mistakenly spelled by *P. scrophulariflora*, the former name is considered to be the accepted (ICBN article, 1908) as a valid name for the eastern Himalayan kutki (mentioned in Rawat *et al*, 2013; Albach *et al.*, 2004a and 2004b; 2005). However, the recent taxonomic revolution based on molecular (DNA sequence) data on this genus does not support the findings of Hooker, 1885; Hong, 1984; and Mill, 2000, and some reshufflings have been carried out. The molecular-based phylogenetic analysis suggested that the evolutionary distance between *Picrorhiza* and *Neopicrorhiza* is not sufficient to establish individual genera and should be treated as sister taxa (Albach *et al.*, 2004a and 2004b; 2005; Pusalkar, 2014). Similarly, the establishment of *P. minima* still remained not to be convincing because except for the shape of anther other all characters are allied to the *P. scrophulariiflora*. To elucidate it, a thorough investigation of molecular structure and taxonomic illustrations based on herbarium specimens is needed.

However, despite this discrepancy in the literature based findings, *Neopicrorhiza scrophulariiflora* is utilized throughout the discussion in this dissertation to support its official records. In this case, genus discussion and identifying keys generated are used from the literature review.

***Picrorhiza*, Royle.**

A clonal rhizomatus herb. Leaves subradical spatulate, serrate. Inflorescence on spicate flowers with leafy peduncle. Flower ebracteolate, ebracteolate, pentamerous, actinomorphic or bilabiate, blueish colour with whitish hairs. Sepals 5, lanceolate and imbricate in bud and persistent. Corolla 5, membranaceous, equal or unequal, often hairy. Stamens 4, slender, short or long. Ovary bi-celled, many-ovuled, stigma simple or capitate. Capsule ovoid and tapering towards acute apex, exposing columnar placentiferous axis, dehiscence septicidal or loculicidal. Seeds oblong enclosed in hyaline reticulated testa.

Keys to the Species

- 1a. Flower usually actinomorphic rarely sub actinomorphic, corolla externally glabrous, stamens far (nearly 10 times) longer than corolla length, not didynamous stamens, Scrofella-type of pollen grains.....
2a
- 1b. Flower zygomorphic, bilabiate, corolla externally glandular ciliate, moderately longer (nearly 3 times) than corolla length, didynamous stamens, Paederota type of pollen grains 2b
- 2a. Comparatively large plant with 30 – 50-flowered spike, corolla tube inconspicuous much shorter than corolla lobes, corolla lobes acute *P. kurrooa*
- 2b. Moderate-sized plant with 5 – 8-flowered spike, corolla tube long and curved
3
- 3a. Corolla lobes assymmetrical, ovate-lanceolate to lanceolate, acutely pointed, with top lip notched to sub-acuminate, lip-apex hooded or galeate with retuse or emarginate, lower lip obliquely placed or sub-erect *P. tungnathii*
- 3b. Uneven corolla, ovate-oblong or obovate with curved tip, upper lip domed or galeate with retuse, emarginate or apiculate apex, lower lip horizontal or reniform (recurved) 4
- 4a. Anther longer than broad, thecae parallel to filament slightly bulging at the base and tapering towards the tip *P. scrophulariiflora*

- 4b. Anther broader than length, theca tips spreading outwards from filament, narrow at the base and broader at the distal end *P. minima*

Conclusive Remarks

The morpho-metric analysis among the species of *Picrorhiza* revealed that species are arranged in two in two clads. *P. kurrooa* lies in one clad and *P. scrophulariiflora*, *P. tungnathii*, and *P. minima* lie in another clad. Flowers having actinomorphic character and shorter stamens with equal length within the corolla tube are the most distinguished characteristics of *P. kurrooa* where as the zygomorphic flower distinguishes the rest three species. *P. tungnathii* possesses more or less equal lengths of all four stamens whereas far longer anterior two stamens and the rest two shorter stamens with equal length to the corolla lobe are striking characters in *P. scrophulariiflora* and *P. minima*. *P. minima* is distinguished due to the presence of anther thecae tips spreading outwards from the filament, narrow at the base and broader at the distal end in compared to thecae parallel to the filament which are slightly bulging at the base and tapering towards the tip in case of *P. scrophulariiflora*. The vegetative stages of all the species virtually possess similar biological traits to each other which could be associated with the trade without distinction. Thus from this study, it is suggested that control and regulation of immature plant harvesting are not only required for the sustainability of plant population but also necessary to address the taxonomic problems.

B. Appendix Tables

Table B.1 List of 19 bioclimatic factors used for predicting *Neopicrorhiza scrophulariiflora* distribution in the Nepalese Himalaya

bio1	Annual mean temperature
bio2*	Mean diurnal range (Mean of monthly (max temp - min temp))
bio3*	Isothermality (bio2/bio7) (* 100)
bio4*	Temperature seasonality (standard deviation *100)
bio5*	Maximum temperature of warmest month
bio6*	Minimum temperature of coldest month
bio7	Temperature annual range (bio5-bio6)
bio8	Mean temperature of wettest quarter
bio9	Mean temperature of driest quarter
bio10	Mean temperature of warmest quarter
bio11	Mean temperature of coldest quarter

bio12	Annual precipitation
bio13	Precipitation of wettest month
bio14	Precipitation of driest month
bio15	Precipitation seasonality (Coefficient of variation)
bio16	Precipitation of wettest quarter
bio17	Precipitation of driest quarter
bio18	Precipitation of warmest quarter
bio19	Precipitation of coldest quarter

*Derived from maximum and minimum temperature

Table B.2 Spearman's correlation coefficient matrix for environmental factors employed for predicting *Neopicrorhiza scrophulariiflora* distribution in the Nepalese Himalaya. Table 2 and Table B.1 both include the variable details

Variables	Elevation	Aspect	Slope	bio1	bio2	bio3	bio4	bio5	bio6	bio7	bio8	bio9	bio10	bio11	bio12	bio13	bio14	bio15	bio16	bio17	bio18	bio19
Elevation	1.00																					
Aspect	-0.05	1.00																				
Slope	-0.09	-0.20	1.00																			
bio1	-0.90	0.09	0.09	1.00																		
bio2	0.41	0.11	-0.11	-0.49	1.00																	
bio3	-0.20	0.14	0.09	0.16	0.46	1.00																
bio4	0.61	0.01	-0.21	-0.68	0.77	-0.19	1.00															
bio5	-0.87	0.10	0.06	0.99	-0.43	0.10	-0.58	1.00														
bio6	-0.87	0.06	0.10	0.98	-0.65	0.04	-0.77	0.96	1.00													
bio7	0.54	0.06	-0.16	-0.61	0.93	0.10	0.94	-0.52	-0.74	1.00												
bio8	-0.89	0.09	0.08	1.00	-0.43	0.17	-0.62	0.98	0.96	-0.55	1.00											
bio9	-0.86	0.03	0.13	0.97	-0.64	0.05	-0.76	0.96	0.99	-0.73	0.95	1.00										
bio10	-0.89	0.10	0.08	1.00	-0.45	0.14	-0.62	0.99	0.96	-0.55	1.00	0.96	1.00									
bio11	-0.89	0.08	0.10	1.00	-0.53	0.16	-0.73	0.98	0.99	-0.66	0.99	0.98	0.99	1.00								
bio12	-0.77	-0.04	0.05	0.77	-0.48	0.12	-0.58	0.72	0.76	-0.58	0.78	0.73	0.77	0.78	1.00							
bio13	-0.76	-0.04	0.03	0.75	-0.37	0.19	-0.52	0.70	0.71	-0.49	0.77	0.69	0.75	0.75	0.99	1.00						
bio14	-0.50	0.05	0.07	0.54	-0.65	-0.36	-0.45	0.51	0.58	-0.56	0.52	0.58	0.53	0.55	0.58	0.47	1.00					
bio15	-0.20	0.13	-0.03	0.15	0.57	0.55	0.22	0.14	-0.02	0.39	0.22	-0.06	0.19	0.12	0.28	0.41	-0.26	1.00				
bio16	-0.76	-0.04	0.04	0.76	-0.38	0.20	-0.53	0.70	0.72	-0.50	0.77	0.69	0.75	0.76	0.99	1.00	0.48	0.39	1.00			
bio17	-0.21	-0.07	0.02	0.29	-0.78	-0.70	-0.38	0.32	0.44	-0.57	0.24	0.44	0.27	0.31	0.24	0.11	0.72	-0.71	0.11	1.00		
bio18	-0.72	-0.05	0.06	0.71	-0.36	0.26	-0.54	0.65	0.68	-0.51	0.73	0.65	0.71	0.72	0.98	0.98	0.41	0.40	0.99	0.05	1.00	
bio19	-0.02	-0.10	-0.01	0.09	-0.71	-0.78	-0.24	0.13	0.26	-0.46	0.04	0.26	0.08	0.12	0.05	-0.07	0.55	-0.79	-0.07	0.96	-0.13	1.00

Table B.4 Measured areas of the various ecological regions of the Nepalese Himalayas' habitat suitability classes (in square kilometers) for *Neopicrorhiza scrophulariiflora*

Ecological region	Low (25–50 %)	Medium (50–75%)	High (>75%)	Total Area (km ²)	Percentage of Nepal's area
Western	2678.41	367.46	0.75	3046.63	2.07
Central	4007.46	1295.16	48.94	5351.56	3.64
Eastern	1756.74	1124.98	336.59	3218.31	2.19
Total	8442.61	2787.6	386.28	11616.5	
Percentage of Nepal's area	5.74	1.89	0.26		7.9

Table B.5 A list of the Nepalese Himalayan districts where *Neopicrorhiza scrophulariiflora* environment is predicted to be suitable

Suitability category	Districts
Highly (as well as medium and low) suitable habitat occurring	Taplejung, Sankhuwasabha, Sindhupalchok, Solukhumbu, Dolakha, Gorkha, Rasuwa, Ramechhap, Manang, Myagdi, Kaski, Dolpa (12 districts)
Medium (and low) suitable habitat occurring	Humla, Mugu, Mustang, Dolakha, Bajhang, Bajura, Jumla, Jajrkot, Rukum West, Rukum East, Baglung, Lamjung, Dhadhing (13 districts)
Only low suitable habitat occurring	Darchula, Kalikot and Panchthar (3 districts)
Total number of districts predicted to include suitable habitats	28

Table B.6 Estimated suitable habitat area (km²) calculated for the protected areas in Himalya Nepal

Protected area	Low	Medium	High	Total
Api-Nampa Conservation Area (ANCA)	39.16	0.00	0.00	39.16
Rara National Park (RNP)	6.02	0.00	0.00	6.02
Sey-Phoksundo National Park (SpNP)	676.95	95.63	0.00	772.58
Dhorpatan Hunting Reserve (DHR)	373.49	60.24	0.00	433.73
Annapurna Conservation Area (ACA)	1381.75	454.81	18.07	1854.63
Manaslu Conservation Area (MCA)	399.09	162.65	2.26	564.00
Langtang National Park (LNP)	408.12	137.80	3.01	548.94
Gaurishankar Conservation Area (GCA)	401.35	212.35	36.14	649.84
Sagarmatha National Park (SNP)	228.16	193.52	84.34	506.01
Makalu Barun National Park (MBCNP)	434.48	268.82	88.10	791.40
Kanchenjunga Conservation Area (KCA)	505.26	339.60	97.14	942.00
Total	4853.83	1925.42	329.06	7108.30

Table B.7 *Neopicrorhiza scrophulariiflora* populations in Nepal's Langtang National Park and Api-Nampa Conservation Area with information on topography, location, climate, and habitat types

Population	Harvest level	Mean elevation (m asl.)	Mean slope in degrees	Aspect	Geographical coordinates		Climate type	Dominant habitat type
Api-Nampa Conservation Area, North-West Nepal								
1. Thadapani	High	3800	39.00	NE-NW	29.97 80.93 E	N,	Lower alpine	Grassland and shrubland meadow
2. Thadeula	Low	4000	36.00	NE-SW	29.97 80.95E	N,	Alpine	Shrubland meadow, Moraine
3. Bainsand	High	4300	45.00	NE-NW	29.95 80.96 E	N,	Alpine	Grassland and shrubland meadow, Moraine
4. Chhanni	Low	4600	46.00	NE	29.95 80.93 E	N,	Upper alpine	Grassland and rocky area
Langtang National Park, North-Central Nepal								
5. Lauribina	High	4000	55.83	NE-NW	28.09 85.37 E	N,	Alpine	Grassland and Shrubland meadow
6. Lauribina Pass	Low	4200	47.83	NW	28.09 85.39 E	N,	Alpine	Shrubland meadow, Moraine
7. Gosainkunda	High	4500	56.67	NE	28.07 85.42 E	N,	Upper alpine	Grassland and Shrubland meadow, Moraine
8. Dudhakunda	Low	4700	46.17	NE	28.09 85.42 E	N,	Upper alpine	Shrubland meadow and rocky boulders

Table B.8 Mean (\pm SD) values of environmental factors among populations of *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area and Langtang National Park, Nepal. Kruskal Wallis tests were used to examine how median variation differed among populations in each region. Significant levels: $p \leq 0.001$ ‘***’, $p \leq 0.01$ ‘**’ and $p \leq 0.05$ ‘*’. Mann-Whitney U tests were used to compare study regions (N = 240).

Environmental variables	Api-Nampa Conservation Area (ANCA)					Langtang National Park (LNP)					Region difference (<i>p</i> value)
	Thadapani (3800 m)	Thadeula (4000 m)	Bainsand (4300 m)	Channi (4600 m)	ANCA overall	Lauribina (4000 m)	Lauribina Pass (4200 m)	Gosainkunda (4500 m)	Dhudhakunda (4700 m)	LNP overall	
Ground cover estimate											
Herb cover %	39.03 \pm 3.37	35.57 \pm 2.95	46.10 \pm 4.07	31.40 \pm 2.05	38.03 \pm 1.65*	29.1 \pm 2.02	22.63 \pm 1.47	35.30 \pm 2.83	12.87 \pm 1.49	24.98 \pm 1.26***	<0.001
Graminoid cover %	15.03 \pm 2.11	8.40 \pm 1.08	9.93 \pm 1.92	15.50 \pm 1.16	12.22 \pm 0.85***	11.63 \pm 1.14	9.43 \pm 0.74	9.50 \pm 0.97	7.53 \pm 1.50	9.53 \pm 0.57***	<0.050
Rock cover %	21.10 \pm 3.07	34.53 \pm 3.45	22.20 \pm 2.76	33.83 \pm 3.14	27.92 \pm 1.64**	39.03 \pm 2.26	41.13 \pm 2.16	35.53 \pm 3.31	54.23 \pm 3.14	42.48 \pm 1.51***	<0.001
Bare ground cover %	5.90 \pm 1.10	9.23 \pm 1.76	10.00 \pm 1.57	11.73 \pm 1.27	9.22 \pm 0.74*	3.13 \pm 0.79	3.20 \pm 0.65	5.47 \pm 1.39	6.33 \pm 1.59	4.53 \pm 0.59	<0.001
Moss/Lichen cover %	18.93 \pm 2.64	12.27 \pm 1.52	11.77 \pm 3.07	7.50 \pm 1.03	12.62 \pm 1.16***	17.07 \pm 1.32	23.60 \pm 1.34	14.20 \pm 1.6	19.03 \pm 2.02	18.48 \pm 0.85***	<0.001
Shrub cover %	20.33 \pm 3.70	10.87 \pm 2.58	10.17 \pm 3.18	2.50 \pm 1.28	10.97 \pm 1.51**	28.73 \pm 2.9	38.43 \pm 1.82	25.23 \pm 3.17	23.70 \pm 3.24	29.03 \pm 1.50**	<0.001
Shrub height m	0.71 \pm 0.07	0.61 \pm 0.05	0.51 \pm 0.10	0.46 \pm 0.14	0.61 \pm 0.04	0.57 \pm 0.02	0.52 \pm 0.02	0.43 \pm 0.02	0.37 \pm 0.02	0.48 \pm 0.01***	<0.010
Solar radiation											
Potential annual direct incident radiation (PADIR)	-0.84 \pm 0.11	-0.77 \pm 0.10	-0.97 \pm 0.10	-0.96 \pm 0.02	-0.88 \pm 0.04**	-1.17 \pm 0.05	-0.97 \pm 0.03	-1.19 \pm 0.05	-0.94 \pm 0.06	-1.07 \pm 0.03***	<0.001
Edaphic factor											
Soil Ph	6.47 \pm 0.05	6.63 \pm 0.03	6.49 \pm 0.04	6.58 \pm 0.04	6.54 \pm 0.02*	6.25 \pm 0.06	6.38 \pm 0.04	6.14 \pm 0.05	6.23 \pm 0.05	6.25 \pm 0.03**	<0.001
Disturbance score											
Grazing effect (0...4)	2.93 \pm 0.19	2.47 \pm 0.16	2.77 \pm 0.14	2.17 \pm 0.16	2.58 \pm 0.08*	2.77 \pm 0.23	2.70 \pm 0.19	2.03 \pm 0.24	1.33 \pm 0.22	2.21 \pm 0.12**	0.049
Harvest effect (0...4)	3.23 \pm 0.22	2.80 \pm 0.25	3.13 \pm 0.20	2.17 \pm 0.21	2.83 \pm 0.12**	2.56 \pm 0.24	1.73 \pm 0.23	1.56 \pm 0.20	1.03 \pm 0.19	1.73 \pm 0.12***	<0.001

Table B.9 Mean (\pm SE) preharvest ramet density (m^{-2}) prior to harvest treatment made in 2015 and their relative measure of cumulative change (annual restoration percentage) in postharvest density of *Neopicrorhiza scrophulariiflora* along the populations in Api-Nampa Conservation Area and Langtang National Park in the Nepalese Himalaya. The plant populations were censused every year between 2015 and 2017 for former and until 2018 for later site.

Response variable (m^{-2})		Harvest treatment (%)	Api-Nampa Conservation Area, north-west Nepal			
			Thadapani (3800 m)	Thadeula (4000 m)	Bainsand (4300 m)	Chhanni (4600 m)
Preharvest 2015	density	0	72.67 \pm 18.81	63.33 \pm 11.86	57.33 \pm 18.67	52.33 \pm 11.68
		25	76.00 \pm 17.94	36.89 \pm 9.28	42.22 \pm 11.22	31.11 \pm 6.17
		50	73.33 \pm 21.18	136.67 \pm 21.36	99.33 \pm 14.53	28.67 \pm 6.36
		75	145.33 \pm 41.59	208.00 \pm 64.37	158.67 \pm 36.25	46.67 \pm 9.33
		100	78.33 \pm 16.05	121.33 \pm 34.64	126.00 \pm 15.14	94.33 \pm 35.62
Density % 2016	restoration	0	12.51 \pm 4.20	7.39 \pm 5.17	9.65 \pm 7.76	6.04 \pm 2.19
		25	-12.42 \pm 10.49	-6.10 \pm 10.71	-8.9 \pm 5.07	-17.16 \pm 1.12
		50	-51.59 \pm 6.25	-49.12 \pm 2.39	-49.83 \pm 2.9	-47.09 \pm 5.91
		75	-81.84 \pm 4.24	-80.48 \pm 0.15	-77.34 \pm 2.92	-73.48 \pm 5.62
		100	-90.26 \pm 1.19	-87.25 \pm 3.00	-89.45 \pm 2.81	-92.40 \pm 1.72
Density % 2017	restoration	0	27.11 \pm 10.96	15.29 \pm 6.43	18.95 \pm 13.08	14.16 \pm 4.87
		25	0.50 \pm 10.83	10.00 \pm 23.63	7.44 \pm 7.65	-14.90 \pm 3.07
		50	-45.40 \pm 7.06	-46.95 \pm 3.23	-44.72 \pm 8.03	-36.11 \pm 7.35
		75	-80.84 \pm 3.33	-77.45 \pm 2.47	-78.93 \pm 2.53	-77.89 \pm 2.62
		100	-86.19 \pm 4.64	-85.39 \pm 2.04	-88.20 \pm 3.72	-87.51 \pm 2.97
Response variable		Harvest treatment (%)	Langtang National Park, north-central Nepal			
			Lauribina (4000 m)	Lauribina Pass (4200 m)	Gosainkunda (4500 m)	Dudhakunda (4700 m)
Preharvest 2015	density	0	97.33 \pm 13.02	84.67 \pm 19.41	97.33 \pm 24.46	72.00 \pm 20.55
		25	57.78 \pm 13.12	45.78 \pm 18.25	32.44 \pm 5.01	48.00 \pm 17.76
		50	49.33 \pm 15.38	62.00 \pm 24.98	79.33 \pm 33.33	55.33 \pm 19.81
		75	78.67 \pm 17.33	102.67 \pm 46.72	182.67 \pm 37.33	142.67 \pm 21.46
		100	73.00 \pm 24.98	127.67 \pm 31.87	88.67 \pm 20.54	207.00 \pm 0.00
Density % 2016	restoration	0	8.59 \pm 1.44	5.98 \pm 3.64	10.83 \pm 2.45	8.28 \pm 2.35
		25	1.21 \pm 10.73	-16.95 \pm 2.25	-10.45 \pm 7.28	-14.99 \pm 4.33
		50	-34.44 \pm 10.97	-36.97 \pm 5.16	-41.17 \pm 5.14	-41.52 \pm 4.77
		75	-74.75 \pm 3.61	-78.63 \pm 4.03	-71.68 \pm 2.13	-71.43 \pm 6.35
		100	-87.17 \pm 4.65	-91.88 \pm 2.03	-84.92 \pm 4.46	-96.14 \pm 0.00
Density % 2017	restoration	0	14.43 \pm 4.26	12.31 \pm 3.69	18.38 \pm 2.65	5.05 \pm 2.53
		25	20.11 \pm 14.22	21.12 \pm 23.83	1.92 \pm 3.59	-9.65 \pm 6.98
		50	-2.87 \pm 11.24	-12.19 \pm 5.56	-13.03 \pm 1.26	-33.43 \pm 4.72
		75	-68.87 \pm 4.40	-75.82 \pm 3.48	-68.53 \pm 2.85	-70.31 \pm 7.28
		100	-77.90 \pm 7.09	-89.70 \pm 1.81	-83.07 \pm 1.46	-88.41 \pm 0.00
Density % 2018	restoration	0	16.24 \pm 3.51	14.78 \pm 5.65	18.96 \pm 4.64	6.53 \pm 2.36
		25	23.28 \pm 11.05	25.87 \pm 21.46	11.96 \pm 6.76	0.92 \pm 14.79
		50	6.64 \pm 16.52	6.64 \pm 11.13	-0.54 \pm 3.00	-28.18 \pm 3.82
		75	-66.28 \pm 3.56	-75.86 \pm 2.46	-69.58 \pm 2.41	-71.30 \pm 7.54
		100	-81.08 \pm 6.39	-89.22 \pm 1.73	-82.47 \pm 0.87	-88.41 \pm 0.00

Table B.10 Repeated-measures ANCOVA for testing the effects of initial density (D0), harvest treatment (H), time (Years: Y) and elevational level (EL) on ramet density (m⁻²) and their reproductive activities per ramet of *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area, north-western Nepal. After applying the experimental harvest treatment (0-100%) at the first year of sampling, two populations at each two different elevation levels (lower alpine: <4250 m and upper alpine: >4250 m) were censused annually between 2015 and 2017. Sampling plot was included as random (subject) factor. The presentation is the extended form of Table 7.

Variable and model parameter		Between-subject Effect					Within-subject effect						
		Density0 (D0)	Harvest treatment (H)	Elevation level (EL)	H*EL	D0*H	Residuals (Plot*H*EL)	Time (Y)	Y*D0	Y*EL	Y*H	Y*EL*H	Residuals (Plot*H*EL*Y)
Density	SS	36.10	476.90	5.30	8.10	42.70	31.50	2.90	37.50	26.00	465.50	7.10	69.70
	Df	1	4	1	4	4	105	1	2	2	8	8	98
	MS	36.13	119.22	5.34	2.02	10.68	0.30	2.89	18.73	13.02	58.18	0.89	0.71
	F	120.28***	396.96**	17.78***	6.73***	35.56***		4.06*	26.33***	18.30***	81.79***	1.26	
Reproductive investment	SS	1.41	71.51	3.79	6.61	2.68	39.38	0	1.41	8.01	67.29	6.61	42.06
	Df	1	3	1	3	3	84	1	2	2	6	6	78
	MS	1.41	23.84	3.79	2.20	0.89	0.47	0	0.71	4.01	11.22	1.10	0.54
	F	3.01	50.84***	8.09**	4.70**	1.90		0	1.31	7.43**	20.80***	2.04	
Fruit abortion%	SS	0.002	0.12	0.005	0.013	0.004	0.13	0.0002	0.002	0.0009	0.11	0.02	0.13
	Df	1	3	1	3	3	84	1	1	2	6	6	79
	MS	0.002	0.04	0.005	0.004	0.001	0.002	0.0002	0.002	0.0005	0.02	0.003	0.002
	F	1.30	23.12***	3.48	2.88*	0.81		0.15	1.25	0.28	11.71***	1.81	
Intact fruit number	SS	1.93	54.71	5.36	7.17	0.97	29.78	4.33	1.93	17.00	45.98	8.37	22.32
	Df	1	3	1	3	3	84	1	1	2	6	6	79
	MS	1.93	18.24	5.36	2.39	0.32	0.36	4.33	1.93	8.50	7.66	1.40	0.28
	F	5.45*	51.44***	15.13***	6.74***	0.91		15.32***	6.84*	30.08***	27.13***	4.94***	
Seed production	SS	12.70	423.50	16.80	44.40	10.40	218.00	24.90	12.70	80.70	376.90	54.90	175.70
	Df	1	3	1	3	3	84	1	1	2	6	6	79
	MS	12.70	141.18	16.76	14.81	3.45	2.59	24.89	12.70	40.37	62.81	9.15	2.22
	F	4.90*	54.41***	6.46*	5.71**	1.33		11.19**	5.71*	18.16***	28.25***	4.15**	
Seed fecundity	SS	0.71	6.64	0.78	1.39	0.14	4.09	0.63	0.71	2.30	5.57	1.53	3.00
	Df	1	3	1	3	3	84	1	1	2	6	6	79
	MS	0.71	2.21	0.78	0.46	0.04	0.05	0.63	0.71	1.15	0.93	0.25	0.04
	F	14.66***	45.50***	15.95***	9.49***	0.92		16.60***	18.79***	30.30***	24.45***	6.71***	
Mortality	SS	14.10	10.18	0.06	1.38.00	1.40	25.13	0.59	15.80	0.16	10.67	4.12	20.93
	Df	1	4	1	4	4	93	1	2	2	7	7	88
	MS	14.10	2.545	0.06	0.35	0.35	0.27	0.59	7.90	0.08	1.52	0.59	0.24
	F	52.17***	9.42***	0.22	1.28	1.29		2.49	33.23***	0.33	6.41***	2.47*	

Levels of significance (*p*) are; ***<0.001, **<0.01, and *<0.05.

Table B.11 Repeated-measures ANCOVA for testing the effects of initial density (D0), harvest treatment (H), time (Years: Y) and elevational level (EL) on ramet density (m⁻²) and their reproductive activities per ramet of *Neopicrorhiza scrophulariiflora* in Langtang National Park, north-central Nepal. After applying the experimental harvest treatment (0-100%) at the first year of sampling, two populations at each two different elevation levels (lower alpine: <4250 m and upper alpine: >4250 m) were censused annually between 2015 and 2018. Sampling plot was included as random (subject) factor. The presentation is the extended form of Table 7. Levels of significance (*p*) are; ***<0.001, **<0.01, and *<0.05.

Variable and model parameter	Between-subject Effect							Within-subject effect					
	Density0 (D0)	Harvest treatment (H)	Elevation level (EL)	H*EL	D0*H	Residuals (Plot*H*EL)	Time (Y)	Y*D0	Y*EL	Y*H	Y*EL*H	Residuals (Plot*H*EL*Y)	
Density	SS	96.80	842.60	1.60	7.70	122.60	89.40	14.30	97.20	2.10	846.50	8.20	192.50
	Df	1	4	1	4	4	159	2	3	3	12	12	141
	MS	96.78	210.65	1.65	1.92	30.65	0.56	7.16	32.39	0.70	70.54	0.68	1.37
	F	172.09***	374.58***	2.93	3.42*	54.51***		5.24**	23.72***	0.511	51.67***	0.50	
Reproductive investment	SS	13.07	85.26	5.86	7.53	4.35	83.83	15.11	13.31	5.17	98.39	10.52	57.4
	Df	1	3	1	3	3	132	2	3	3	9	9	117
	MS	13.07	28.42	5.86	2.51	1.45	0.64	7.55	4.44	1.72	10.93	1.17	0.49
	F	20.59***	44.75***	9.23**	3.95**	2.28		15.40***	9.04***	3.51*	22.29***	2.38*	
Fruit abortion%	SS	0.0004	0.09	0.0006	0.02	0.01	0.16	0.007	0.00043	0.007	0.11	0.02	0.13
	Df	1	3	1	3	3	132	2	1	3	9	9	119
	MS	0.0004	0.03	0.0006	0.005	0.003	0.001	0.004	0.0004	0.002	0.01	0.002	0.001
	F	0.36	25.35***	0.53	4.27**	2.97*		3.32*	0.39	2.13	10.79***	1.96	
Intact fruit number	SS	14.40	59.20	3.47	5.21	1.79	63.10	12.71	14.40	3.90	66.75	7.86	41.56
	Df	1	3	1	3	3	132	2	1	3	9	9	119
	MS	14.40	19.74	3.47	1.74	0.60	0.48	6.35	14.40	1.30	7.42	0.87	0.35
	F	30.13***	41.28***	7.26**	3.63*	1.25		18.19***	41.23***	3.72*	21.23***	2.50*	
Seed production	SS	101.30	471.60	8.60	32.60	10.70	463.40	85.60	101.30	12.80	539.90	52.00	296.60
	Df	1	3	1	3	3	132	2	1	3	9	9	119
	MS	101.27	157.20	8.57	10.87	3.58	3.51	42.79	101.27	4.26	59.99	5.78	2.49
	F	28.85***	44.78***	2.44	3.10*	1.02		17.17***	40.63***	1.71	24.07	2.32*	
Seed fecundity	SS	6.98	15.70	1.08	1.91	0.44	18.22	3.05	6.98	1.15	17.86	2.64	12.66
	Df	1	3	1	3	3	132	2	1	3	9	9	119
	MS	6.98	5.23	1.08	0.64	0.15	0.14	1.52	6.99	0.38	1.98	0.29	0.11
	F	50.59***	37.90***	7.83**	4.61**	1.07		14.32***	65.65***	3.59*	18.65***	2.76**	
Mortality	SS	15.92	4.17	2.46	2.57	8.60	46.21	2.19	17.5	3.53	8.70	5.28	42.72
	Df	1	4	1	4	4	149	2	3	3	11	11	133
	MS	15.92	1.04	2.46	0.64	2.15	0.31	1.10	5.83	1.18	0.79	0.48	0.32
	F	51.33***	3.36*	7.92**	2.07	6.93***		3.41*	18.16***	3.66*	2.46**	1.495	

Table B.12 Mean (\pm SE) reproductive activities of *Neopicrorhiza scrophulariiflora* per ramet censused prior to experimental harvest on density (m^{-2}) applied in 2015 and their relative change (restoration %) in a two-years of postharvest-period along with populations in Api-Nampa Conservation Area in north-western Himalaya Nepal. Variables are further explained in Figure C.3.

Response variable	Harvest treatment (%)	Thadapani (3800 m)			Thadeula (4000 m)			Bainsand (4300 m)			Channi (4600 m)		
		Preharvest 2015	Restoration % 2016	Restoration % 2017	Preharvest 2015	Restoration % 2016	Restoration % 2017	Preharvest 2015	Restoration % 2016	Restoration % 2017	Preharvest 2015	Restoration % 2016	Restoration % 2017
Reproductive investment	0	7.48 \pm 0.74	32.49 \pm 35.62	87.64 \pm 40.56	12.55 \pm 2.18	-8.54 \pm 11.77	23.30 \pm 15.29	5.19 \pm 0.55	83.63 \pm 32.89	79.84 \pm 25.74	5.77 \pm 0.87	18.75 \pm 60.70	36.30 \pm 14.49
	25	7.93 \pm 1.48	-66.67 \pm 33.33	78.6 \pm 48.7	10.46 \pm 1.9	30.69 \pm 28.82	28.33 \pm 19.09	6.89 \pm 0.56	-68.42 \pm 31.58	42.22 \pm 15.29	6.39 \pm 1.15	-100.00 \pm 0.00	-100.00 \pm 0.00
	50	10.23 \pm 0.99	-72.68 \pm 27.32	-62.96 \pm 37.04	11.74 \pm 0.86	-100.00 \pm 0.00	33.22 \pm 23.82	6.83 \pm 0.17	-100.00 \pm 0.00	-100.00 \pm 0.00	4.39 \pm 0.93	-100.00 \pm 0.00	-100.00 \pm 0.00
	75	11.64 \pm 1.2	-100.00 \pm 0.00	-100.00 \pm 0.00	13.03 \pm 0.23	-100.00 \pm 0.00	-100.00 \pm 0.00	6.13 \pm 0.55	-100.00 \pm 0.00	-100.00 \pm 0.00	3.53 \pm 0.46	-100.00 \pm 0.00	-100.00 \pm 0.00
Fruit abortion	0	48.27 \pm 2.4	-16.26 \pm 5.52	-25.91 \pm 6.93	32.86 \pm 3.88	-8.07 \pm 12.39	-6.81 \pm 10.59	46.67 \pm 4.98	13.48 \pm 5.55	-6.90 \pm 8.15	44.81 \pm 0.9	-12.31 \pm 17.31	4.38 \pm 7.15
	25	41.04 \pm 4.57	-28.7 \pm 14.47	-26.48 \pm 6.84	28.9 \pm 5.29	4.51 \pm 12.71	-12.34 \pm 7.39	44.41 \pm 3.99	-16.63 \pm 31.45	3.97 \pm 7.04	43.55 \pm 2.73	-43.55 \pm 2.73	-43.55 \pm 2.73
	50	26.19 \pm 2.2	-7.44 \pm 18.65	-6.19 \pm 18.27	28.76 \pm 1.16	-28.76 \pm 1.16	-5.45 \pm 8.25	51.06 \pm 4.47	-51.06 \pm 4.47	-51.06 \pm 4.47	48.43 \pm 7.61	-48.43 \pm 7.61	-48.43 \pm 7.61
	75	23.65 \pm 2.69	-23.65 \pm 2.69	-23.65 \pm 2.69	22.13 \pm 1.65	-22.13 \pm 1.65	-22.13 \pm 1.65	41.61 \pm 1.95	-41.61 \pm 1.95	-41.61 \pm 1.95	41.81 \pm 4.28	-41.81 \pm 4.28	-41.81 \pm 4.28
Intact fruit setting	0	3.77 \pm 0.56	67.41 \pm 38.76	175.76 \pm 66.61	7.15 \pm 1.59	24.58 \pm 29.04	62.03 \pm 43.06	2.69 \pm 0.36	41.98 \pm 29.53	111.33 \pm 46.99	2.98 \pm 0.53	13.64 \pm 57.20	8.15 \pm 12.58
	25	4.43 \pm 0.99	-71.26 \pm 28.74	171.38 \pm 89.35	7.55 \pm 1.7	27.1 \pm 26.73	39.39 \pm 31.24	3.6 \pm 0.31	-91.67 \pm 8.33	19.76 \pm 20.25	3.17 \pm 0.44	-100.00 \pm 0.00	-100.00 \pm 0.00
	50	7.01 \pm 0.64	-81.71 \pm 18.29	-77.78 \pm 22.22	7.79 \pm 0.64	-100.00 \pm 0.00	8.34 \pm 17.58	3.43 \pm 0.23	-100.00 \pm 0.00	-100.00 \pm 0.00	2.46 \pm 0.54	-100.00 \pm 0.00	-100.00 \pm 0.00
	75	7.5 \pm 0.76	-100.00 \pm 0.00	-100.00 \pm 0.00	9.23 \pm 0.29	-100.00 \pm 0.00	-100.00 \pm 0.00	3.44 \pm 0.34	-100.00 \pm 0.00	-100.00 \pm 0.00	1.97 \pm 0.32	-100.00 \pm 0.00	-100.00 \pm 0.00
Seed production	0	153.81 \pm 22.89	65.23 \pm 38.26	160.23 \pm 62.86	311.54 \pm 69.23	24.38 \pm 28.99	57.94 \pm 41.97	115 \pm 15.47	40.76 \pm 29.27	104.56 \pm 45.49	97.16 \pm 17.34	8.17 \pm 54.44	-0.37 \pm 11.58
	25	180.88 \pm 40.55	-71.64 \pm 28.36	156.08 \pm 84.32	328.71 \pm 74.19	26.91 \pm 26.69	35.87 \pm 30.45	153.72 \pm 13.05	-91.74 \pm 8.26	15.93 \pm 19.6	103.13 \pm 14.36	-100.00 \pm 0.00	-100.00 \pm 0.00
	50	286.05 \pm 26.16	-81.95 \pm 18.05	-79.03 \pm 20.97	339.51 \pm 27.78	-100.00 \pm 0.00	5.6 \pm 17.14	146.29 \pm 9.72	-100.00 \pm 0.00	-100.00 \pm 0.00	79.97 \pm 17.73	-100.00 \pm 0.00	-100.00 \pm 0.00
	75	306 \pm 31.16	-100.00 \pm 0.00	-100.00 \pm 0.00	402.02 \pm 12.8	-100.00 \pm 0.00	-100.00 \pm 0.00	147.08 \pm 14.49	-100.00 \pm 0.00	-100.00 \pm 0.00	64.23 \pm 10.43	-100.00 \pm 0.00	-100.00 \pm 0.00
Fecundity	0	0.58 \pm 0.09	65.18 \pm 38.22	160.16 \pm 62.88	1.17 \pm 0.26	24.39 \pm 29.01	57.92 \pm 41.97	0.43 \pm 0.06	40.78 \pm 29.34	104.6 \pm 45.6	0.36 \pm 0.07	8.07 \pm 54.4	-0.37 \pm 11.57
	25	0.68 \pm 0.15	-71.63 \pm 28.37	155.91 \pm 84.13	1.23 \pm 0.28	26.89 \pm 26.69	35.89 \pm 30.46	0.58 \pm 0.05	-91.73 \pm 8.27	15.96 \pm 19.64	0.39 \pm 0.05	-100.00 \pm 0.00	-100.00 \pm 0.00
	50	1.07 \pm 0.1	-81.94 \pm 18.06	-79.01 \pm 20.99	1.27 \pm 0.1	-100.00 \pm 0.00	5.61 \pm 17.14	0.55 \pm 0.04	-100 \pm 0.00	-100.00 \pm 0.00	0.3 \pm 0.07	-100.00 \pm 0.00	-100.00 \pm 0.00
	75	1.15 \pm 0.12	-100.00 \pm 0.00	-100.00 \pm 0.00	1.51 \pm 0.05	-100.00 \pm 0.00	-100.00 \pm 0.00	0.55 \pm 0.05	-100.00 \pm 0.00	-100.00 \pm 0.00	0.24 \pm 0.04	-100.00 \pm 0.00	-100.00 \pm 0.00

Table B.13 Mean (\pm SE) reproductive activities of *Neopicrorhiza scrophulariiflora* per ramet censused prior to experimental harvest on density (m^{-2}) applied in 2015 and their relative change (restoration %) in a three-years of postharvest-period along with populations in Langtang National Park, north-central Himalaya Nepal. Variables are further explained in Figure C.4.

Response variable	Harvest treatment (%)	Lauribina (4000 m)				Lauribina Pass (4200 m)				Gosainkunda (4500 m)				Dudhakunda (4700 m)			
		Preharvest value (2015)	Restoration %			Preharvest value (2015)	Restoration %			Preharvest value (2015)	Restoration %			Preharvest value (2015)	Restoration %		
			2016	2017	2018		2016	2017	2018		2016	2017	2018		2016	2017	2018
Reproductive investment	0	11.11 \pm 1.45	-7.96 \pm 10.32	18.83 \pm 26.16	18.98 \pm 31.65	11.88 \pm 1.70	-6.17 \pm 10.20	-15.75 \pm 7.69	-22.04 \pm 4.91	13.51 \pm 1.11	-30.77 \pm 3.09	-9.66 \pm 24.33	-9.93 \pm 17.28	10.03 \pm 0.85	-5.12 \pm 7.08	-16.29 \pm 14.66	-3.11 \pm 1.75
	25	10.97 \pm 0.30	-15.16 \pm 6.10	48.28 \pm 8.70	16.07 \pm 9.89	11.33 \pm 2.33	13.67 \pm 27.28	13.49 \pm 22.87	5.61 \pm 24.42	8.61 \pm 1.88	7.46 \pm 22.53	10.67 \pm 26.96	45.49 \pm 73.18	8.17 \pm 0.54	-100.00 \pm 0.00	-37.21 \pm 62.79	8.55 \pm 39.24
	50	12.18 \pm 0.10	-63.51 \pm 36.49	-8.38 \pm 4.63	3.59 \pm 20.45	13.02 \pm 0.18	-73.83 \pm 26.17	-25.15 \pm 13.08	-33.16 \pm 7.4	9.39 \pm 1.20	-100.00 \pm 0.00	-36.35 \pm 63.65	-53.32 \pm 46.68	7.49 \pm 0.25	-100.00 \pm 0.00	-60.87 \pm 39.13	-76.09 \pm 23.91
	75	12.62 \pm 1.73	-100 \pm 0.00	-100.00 \pm 0.00	-57.04 \pm 23.91	11.20 \pm 1.91	-100.00 \pm 0.00	-100.00 \pm 0.00	-38.61 \pm 31.48	9.83 \pm 1.73	-100.00 \pm 0.00	-100.00 \pm 0.00	-53.17 \pm 27.37	8.06 \pm 0.73	-100.00 \pm 0.00	-100.00 \pm 0.00	-53.49 \pm 46.51
Fruit abortion	0	32.12 \pm 4.86	15.31 \pm 28.00	-21.41 \pm 14.37	-25.31 \pm 25.87	26.87 \pm 3.21	2.37 \pm 39.60	8.21 \pm 15.57	-35.27 \pm 2.39	28.70 \pm 3.85	-17.32 \pm 5.38	-4.74 \pm 16.43	-13.75 \pm 15.77	40.89 \pm 2.53	36.59 \pm 14.75	50.67 \pm 18.80	12.74 \pm 17.47
	25	30.10 \pm 1.66	-8.59 \pm 6.23	-26.18 \pm 11.61	-32.17 \pm 3.64	28.97 \pm 5.36	-19.71 \pm 12.43	-6.38 \pm 12.36	-38.71 \pm 17.53	38.97 \pm 1.4	-56.59 \pm 23.89	-22.04 \pm 3.13	-51.94 \pm 2.40	57.08 \pm 2.64	-100.00 \pm 0.00	-70.05 \pm 29.95	-28.22 \pm 1.13
	50	20.58 \pm 5.30	-33.82 \pm 66.18	-2.99 \pm 33.94	22.03 \pm 31.05	23.28 \pm 2.07	-64.31 \pm 35.69	30.6 \pm 39.94	-27.72 \pm 6.17	41.86 \pm 2.97	-100.00 \pm 0.00	-62.27 \pm 37.73	-56.52 \pm 43.48	63.89 \pm 1.41	-100.00 \pm 0.00	-59.6 \pm 40.4	-80.57 \pm 19.43
	75	23.56 \pm 3.26	-100.00 \pm 0.00	-100.00 \pm 0.00	-37.89 \pm 33.6	26.11 \pm 4.08	-100.00 \pm 0.00	-100.00 \pm 0.00	-60.79 \pm 21.49	42.09 \pm 2.61	-100.00 \pm 0.00	-100.00 \pm 0.00	-64.02 \pm 21.23	52.72 \pm 6.01	-100.00 \pm 0.00	-100.00 \pm 0.00	-68.95 \pm 31.05
Intact fruit setting	0	6.62 \pm 0.64	-4.44 \pm 16.39	30.30 \pm 21.13	30.75 \pm 21.11	8.30 \pm 0.91	-0.12 \pm 13.35	-17.74 \pm 16.24	-12.16 \pm 7.26	7.99 \pm 0.50	-31.66 \pm 8.74	-5.34 \pm 25.52	1.59 \pm 15.26	5.97 \pm 0.69	-36.45 \pm 5.38	-45.72 \pm 21.01	-16.66 \pm 11.00
	25	7.21 \pm 0.19	-7.21 \pm 4.74	33.27 \pm 17.11	33.42 \pm 10.17	8.20 \pm 2.17	26.17 \pm 34.08	10.5 \pm 34.19	20.30 \pm 26.32	5.00 \pm 1.32	15.72 \pm 29.32	40.33 \pm 43.23	94.18 \pm 103.2	3.61 \pm 0.28	-100.00 \pm 0.00	-29.99 \pm 70.01	12.67 \pm 27.82
	50	9.32 \pm 0.80	-70.69 \pm 29.31	-0.39 \pm 13.18	6.05 \pm 29.11	9.85 \pm 0.29	-73.33 \pm 26.67	-31.42 \pm 14.39	-27.24 \pm 8.49	5.17 \pm 0.87	-100.00 \pm 0.00	-42.14 \pm 57.86	-44.55 \pm 55.45	2.83 \pm 0.10	-100.00 \pm 0.00	-76.47 \pm 23.53	-58.82 \pm 41.18
	75	9.21 \pm 0.72	-100.00 \pm 0.00	-100.00 \pm 0.00	-54.50 \pm 23.96	8.42 \pm 1.94	-100.00 \pm 0.00	-100.00 \pm 0.00	-27.46 \pm 36.41	4.84 \pm 1.05	-100.00 \pm 0.00	-100.00 \pm 0.00	-42.73 \pm 33.07	3.89 \pm 0.63	-100.00 \pm 0.00	-100.00 \pm 0.00	-50.01 \pm 49.99
Seed production	0	324.91 \pm 31.40	-5.67 \pm 16.18	26.58 \pm 20.53	27.28 \pm 20.55	412.79 \pm 45.13	-3.20 \pm 12.94	-23.53 \pm 15.10	-21.75 \pm 6.46	391.34 \pm 24.68	-34.54 \pm 8.37	-10.94 \pm 24.01	-8.02 \pm 13.82	186.53 \pm 21.48	-39.09 \pm 5.15	-19.94 \pm 30.99	-22.44 \pm 10.24
	25	354.01 \pm 9.45	-8.41 \pm 4.68	29.47 \pm 16.62	29.89 \pm 9.90	407.81 \pm 108.09	22.28 \pm 33.03	2.73 \pm 31.78	7.16 \pm 23.44	244.72 \pm 64.8	10.84 \pm 28.08	32.01 \pm 40.67	75.81 \pm 93.44	112.79 \pm 8.68	-100.00 \pm 0.00	3.24 \pm 103.24	4.85 \pm 25.89
	50	457.41 \pm 39.17	-71.07 \pm 28.93	-3.23 \pm 12.8	3.24 \pm 28.34	489.87 \pm 14.41	-74.16 \pm 25.84	-36.25 \pm 13.38	-35.19 \pm 7.57	253.2 \pm 42.74	-100.00 \pm 0.00	-45.56 \pm 54.44	-49.8 \pm 50.21	88.49 \pm 3.01	-100.00 \pm 0.00	-65.3 \pm 34.70	-61.68 \pm 38.32
	75	452.11 \pm 35.45	-100.00 \pm 0.00	-100.00 \pm 0.00	-55.71 \pm 23.32	418.59 \pm 96.40	-100.00 \pm 0.00	-100.00 \pm 0.00	-35.39 \pm 32.43	237.13 \pm 51.38	-100.00 \pm 0.00	-100.00 \pm 0.00	-48.14 \pm 29.94	121.46 \pm 19.61	-100.00 \pm 0.00	-100.00 \pm 0.00	-53.47 \pm 46.53
Fecundity	0	2.50 \pm 0.19	-5.65 \pm 16.18	26.59 \pm 20.55	27.29 \pm 20.55	1.98 \pm 0.07	-3.22 \pm 12.93	-23.53 \pm 15.08	-21.76 \pm 6.45	2.23 \pm 0.29	-34.52 \pm 8.37	-10.94 \pm 24.01	-8.01 \pm 13.81	0.92 \pm 0.19	-39.08 \pm 5.14	-19.91 \pm 31.02	-22.42 \pm 10.25
	25	2.86 \pm 0.14	-8.42 \pm 4.67	29.45 \pm 16.61	29.89 \pm 9.89	2.42 \pm 0.03	22.26 \pm 33.02	2.72 \pm 31.77	7.13 \pm 23.42	2.31 \pm 0.88	10.87 \pm 28.09	32.05 \pm 40.67	75.82 \pm 93.45	0.72 \pm 0.15	-100.00 \pm 0.00	3.17 \pm 103.17	4.83 \pm 25.83
	50	2.83 \pm 0.53	-71.06 \pm 28.94	-3.22 \pm 12.80	3.23 \pm 28.33	1.97 \pm 0.20	-74.16 \pm 25.84	-36.25 \pm 13.38	-35.18 \pm 7.56	1.17 \pm 0.62	-100.00 \pm 0.00	-45.58 \pm 54.42	-49.81 \pm 50.19	0.21 \pm 0.21	-100.00 \pm 0.00	-65.28 \pm 34.72	-61.66 \pm 38.34
	75	1.22 \pm 0.61	-100.00 \pm 0.00	-100.00 \pm 0.00	-55.69 \pm 23.33	1.31 \pm 0.67	-100.00 \pm 0.00	-100.00 \pm 0.00	-35.38 \pm 32.43	0.74 \pm 0.74	-100.00 \pm 0.00	-100.00 \pm 0.00	-30.87 \pm 34.57	0.24 \pm 0.24	-100.00 \pm 0.00	-100.00 \pm 0.00	-53.49 \pm 46.51

Table B.14 Sensitivity matrices for each of the two *Neopicrorhiza scrophulariiflora* population types (lower alpine and upper alpine) along the harvesting treatments in Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), censused in two transition years (2015–2017) for former and three transition years 2015–2018 for later.

Transition		Api-Nampa Conservation Area (ANCA)															
		0% (control)								25%							
		Lower alpine (<4200 m)				Upper alpine (>4200 m)				Lower alpine (<4200 m)				Upper alpine (>4200 m)			
Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	
2015-16	SP	0.181	0.135	0.114	0.108	0.114	0.124	0.133	0.136	0.157	0.113	0.098	0.095	0.075	0.078	0.082	0.082
	JV	0.555	0.415	0.349	0.331	0.297	0.324	0.348	0.356	0.620	0.444	0.386	0.374	0.258	0.269	0.282	0.284
	AV	0.402	0.300	0.253	0.240	0.359	0.391	0.420	0.430	0.479	0.343	0.298	0.289	0.507	0.528	0.554	0.558
	Rep	0.253	0.189	0.159	0.151	0.119	0.129	0.139	0.142	0.167	0.120	0.104	0.101	0.093	0.097	0.101	0.102
2016-17	SP	0.150	0.109	0.088	0.085	0.085	0.093	0.095	0.096	0.211	0.148	0.123	0.110	0.147	0.146	0.146	0.146
	JV	0.383	0.278	0.225	0.216	0.418	0.458	0.468	0.469	0.346	0.242	0.201	0.179	0.349	0.348	0.348	0.348
	AV	0.677	0.492	0.398	0.382	0.332	0.364	0.372	0.373	0.649	0.455	0.378	0.337	0.452	0.451	0.451	0.450
	Rep	0.308	0.223	0.181	0.173	0.075	0.082	0.084	0.084	0.324	0.227	0.189	0.168	0.055	0.055	0.055	0.055
Transition		50%								75%							
		Lower alpine (<4200 m)				Upper alpine (>4200 m)				Lower alpine (<4200 m)				Upper alpine (>4200 m)			
		Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV
2015-16	SP	0.069	0.115	0.131	0.140	0.166	0.183	0.157	0.000	0.176	0.332	0.206	0.000	0.133	0.150	0.157	0.000
	JV	0.336	0.556	0.636	0.676	0.524	0.579	0.497	0.000	0.253	0.477	0.297	0.000	0.316	0.356	0.375	0.000
	AV	0.171	0.283	0.324	0.344	0.269	0.297	0.255	0.000	0.296	0.558	0.347	0.000	0.431	0.486	0.511	0.000
	Rep	0.025	0.042	0.048	0.051	0.031	0.034	0.029	0.000	0.221	0.416	0.259	0.000	0.012	0.014	0.014	0.000
2016-17	SP	0.174	0.157	0.146	0.143	0.257	0.231	0.218	0.000	0.143	0.171	0.202	0.000	0.276	0.292	0.299	0.000
	JV	0.306	0.276	0.257	0.252	0.369	0.331	0.313	0.000	0.189	0.226	0.267	0.000	0.418	0.442	0.452	0.000
	AV	0.520	0.470	0.438	0.429	0.485	0.435	0.412	0.000	0.446	0.536	0.631	0.000	0.260	0.276	0.282	0.000
	Rep	0.136	0.123	0.114	0.112	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Transition		Langtang National Park (LNP)															
		0% (control)								25%							
		Lower alpine (<4200 m)				Upper alpine (>4200 m)				Lower alpine (<4200 m)				Upper alpine (>4200 m)			
Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	
2015-16	SP	0.109	0.057	0.048	0.046	0.067	0.052	0.050	0.049	0.112	0.056	0.045	0.044	0.078	0.063	0.061	0.059
	JV	0.634	0.328	0.279	0.268	0.385	0.299	0.289	0.284	0.802	0.400	0.324	0.319	0.292	0.236	0.226	0.222
	AV	0.928	0.480	0.409	0.392	0.682	0.530	0.512	0.504	0.884	0.440	0.357	0.351	0.744	0.602	0.576	0.565
	Rep	0.364	0.188	0.160	0.154	0.166	0.129	0.124	0.122	0.331	0.165	0.134	0.132	0.144	0.116	0.111	0.109
2016-17	SP	0.168	0.099	0.084	0.075	0.112	0.078	0.069	0.067	0.286	0.137	0.116	0.084	0.188	0.143	0.131	0.123
	JV	0.332	0.194	0.166	0.149	0.384	0.268	0.237	0.231	0.304	0.146	0.123	0.089	0.240	0.183	0.167	0.157
	AV	0.922	0.540	0.461	0.413	0.785	0.547	0.485	0.472	0.984	0.472	0.400	0.288	0.672	0.512	0.469	0.439
	Rep	0.394	0.231	0.197	0.176	0.225	0.157	0.139	0.135	0.575	0.276	0.234	0.168	0.246	0.187	0.172	0.161
2017-18	SP	0.133	0.071	0.064	0.060	0.091	0.064	0.057	0.055	0.220	0.144	0.108	0.090	0.191	0.139	0.113	0.105
	JV	0.286	0.154	0.137	0.129	0.234	0.165	0.148	0.143	0.132	0.086	0.065	0.054	0.334	0.244	0.198	0.184
	AV	1.149	0.618	0.552	0.520	0.965	0.679	0.608	0.588	0.951	0.622	0.465	0.389	0.676	0.494	0.401	0.373
	Rep	0.357	0.192	0.172	0.161	0.224	0.157	0.141	0.136	0.559	0.365	0.273	0.229	0.298	0.218	0.177	0.165
Transition		50%								75%							
		Lower alpine (<4200 m)				Upper alpine (>4200 m)				Lower alpine (<4200 m)				Upper alpine (>4200 m)			
		Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV
2015-16	SP	0.222	0.138	0.108	0.104	0.077	0.089	0.076	0.000	0.117	0.112	0.098	0.000	0.136	0.114	0.096	0.000
	JV	0.618	0.385	0.300	0.290	0.356	0.409	0.350	0.000	0.471	0.451	0.392	0.000	0.373	0.312	0.263	0.000
	AV	0.622	0.387	0.302	0.292	0.524	0.601	0.514	0.000	0.519	0.497	0.432	0.000	0.785	0.656	0.552	0.000
	Rep	0.194	0.121	0.094	0.091	0.082	0.094	0.080	0.000	0.040	0.038	0.033	0.000	0.086	0.072	0.061	0.000
2016-17	SP	0.357	0.187	0.131	0.089	0.233	0.177	0.162	0.149	0.304	0.272	0.257	0.000	0.338	0.394	0.410	0.000
	JV	0.409	0.215	0.150	0.102	0.236	0.179	0.164	0.151	0.192	0.172	0.163	0.000	0.177	0.207	0.215	0.000
	AV	0.825	0.434	0.303	0.207	0.753	0.571	0.523	0.483	0.619	0.555	0.524	0.000	0.375	0.438	0.456	0.000
	Rep	0.501	0.263	0.184	0.126	0.101	0.077	0.070	0.065	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2017-18	SP	0.294	0.181	0.138	0.103	0.237	0.209	0.180	0.175	0.305	0.203	0.209	0.169	0.169	0.193	0.217	0.227
	JV	0.246	0.152	0.116	0.087	0.263	0.233	0.200	0.194	0.194	0.129	0.132	0.107	0.167	0.190	0.214	0.223
	AV	0.763	0.471	0.358	0.268	0.541	0.478	0.411	0.398	0.572	0.381	0.391	0.317	0.407	0.464	0.522	0.545
	Rep	0.557	0.344	0.262	0.196	0.162	0.143	0.123	0.119	0.315	0.210	0.216	0.175	0.088	0.100	0.113	0.118

Table B.15 Elasticity matrices for each of the two *Neopicrorhiza scrophulariiflora* population types (lower alpine and upper alpine) along the harvesting treatments in Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), censused in two transition years (2015–2017) for former and three transition years 2015–2018 for later.

Api-Nampa Conservation Area (ANCA)																	
Transition	0%									25%							
	Lower alpine (<4250 m)				Upper alpine (>4250 m)					Lower alpine (<4250 m)				Upper alpine (>4250 m)			
	Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep
2015-16	SP	0.000	0.016	0.054	0.111	0.000	0.009	0.043	0.063	0.000	0.003	0.080	0.073	0.000	0.008	0.065	0.002
	JV	0.165	0.231	0.019	0.000	0.109	0.179	0.036	0.000	0.146	0.255	0.036	0.008	0.075	0.167	0.027	0.000
	AV	0.016	0.127	0.070	0.040	0.005	0.136	0.200	0.079	0.011	0.149	0.118	0.020	0.000	0.094	0.360	0.100
	Rep	0.000	0.041	0.110	0.000	0.000	0.000	0.142	0.000	0.000	0.037	0.064	0.000	0.000	0.000	0.102	0.000
2016-17	SP	0.000	0.010	0.020	0.120	0.000	0.005	0.022	0.058	0.000	0.011	0.059	0.141	0.000	0.019	0.078	0.050
	JV	0.132	0.087	0.059	0.000	0.080	0.266	0.113	0.000	0.129	0.085	0.026	0.002	0.116	0.190	0.042	0.000
	AV	0.018	0.143	0.184	0.054	0.005	0.153	0.187	0.027	0.082	0.118	0.154	0.025	0.030	0.132	0.283	0.005
	Rep	0.000	0.037	0.136	0.000	0.000	0.034	0.051	0.000	0.000	0.028	0.140	0.000	0.000	0.008	0.047	0.000
Transition	50%									75%							
	Lower alpine (<4250 m)				Upper alpine (>4250 m)					Lower alpine (<4250 m)				Upper alpine (>4250 m)			
	Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep
2015-16	SP	0.000	0.001	0.052	0.016	0.000	0.079	0.087	0.000	0.000	0.050	0.126	0.000	0.000	0.062	0.071	0.000
	JV	0.062	0.448	0.045	0.000	0.152	0.376	0.051	0.000	0.037	0.244	0.197	0.000	0.133	0.171	0.052	0.000
	AV	0.008	0.106	0.175	0.035	0.014	0.124	0.116	0.000	0.139	0.183	0.025	0.000	0.000	0.123	0.388	0.000
	Rep	0.000	0.000	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2016-17	SP	0.000	0.003	0.063	0.108	0.000	0.111	0.146	0.000	0.000	0.006	0.137	0.000	0.000	0.128	0.148	0.000
	JV	0.136	0.099	0.041	0.000	0.170	0.116	0.045	0.000	0.107	0.073	0.046	0.000	0.208	0.220	0.014	0.000
	AV	0.037	0.147	0.250	0.004	0.087	0.104	0.221	0.000	0.035	0.148	0.448	0.000	0.068	0.094	0.119	0.000
	Rep	0.000	0.028	0.084	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Langtang National Park (LNP)																	
Transition	0% (control)									25%							
	Lower alpine (<4250 m)				Upper alpine (>4250 m)					Lower alpine (<4250 m)				Upper alpine (>4250 m)			
	Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep
2015-16	SP	0.000	0.005	0.012	0.092	0.000	0.002	0.009	0.056	0.000	0.008	0.029	0.075	0.000	0.009	0.025	0.044
	JV	0.084	0.155	0.089	0.000	0.042	0.148	0.109	0.000	0.099	0.226	0.074	0.000	0.046	0.100	0.091	0.000
	AV	0.026	0.131	0.191	0.061	0.025	0.137	0.283	0.067	0.012	0.120	0.168	0.057	0.033	0.128	0.351	0.065
	Rep	0.000	0.038	0.116	0.000	0.000	0.012	0.111	0.000	0.000	0.045	0.086	0.000	0.000	0.000	0.109	0.000
2016-17	SP	0.000	0.008	0.013	0.147	0.000	0.005	0.007	0.100	0.000	0.045	0.092	0.149	0.000	0.017	0.033	0.138
	JV	0.078	0.066	0.051	0.000	0.078	0.114	0.076	0.000	0.092	0.024	0.030	0.000	0.081	0.050	0.051	0.000
	AV	0.090	0.097	0.244	0.029	0.034	0.117	0.299	0.035	0.194	0.060	0.127	0.019	0.106	0.081	0.258	0.023
	Rep	0.000	0.024	0.152	0.000	0.000	0.032	0.103	0.000	0.000	0.016	0.152	0.000	0.000	0.034	0.126	0.000
2017-18	SP	0.000	0.003	0.007	0.123	0.000	0.002	0.005	0.083	0.000	0.006	0.020	0.194	0.000	0.026	0.022	0.143
	JV	0.047	0.039	0.067	0.000	0.046	0.059	0.060	0.000	0.060	0.013	0.014	0.000	0.128	0.081	0.035	0.000
	AV	0.086	0.063	0.364	0.039	0.045	0.094	0.417	0.053	0.160	0.044	0.226	0.035	0.063	0.101	0.215	0.022
	Rep	0.000	0.048	0.114	0.000	0.000	0.010	0.126	0.000	0.000	0.023	0.205	0.000	0.000	0.036	0.128	0.000
Transition	50%									75%							
	Lower alpine (<4250 m)				Upper alpine (>4250 m)					Lower alpine (<4250 m)				Upper alpine (>4250 m)			
	Stage	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep	SP	JV	AV	Rep
2015-16	SP	0.000	0.048	0.103	0.072	0.000	0.019	0.058	0.000	0.000	0.023	0.094	0.000	0.000	0.048	0.088	0.000
	JV	0.188	0.154	0.043	0.000	0.060	0.172	0.177	0.000	0.109	0.251	0.091	0.000	0.076	0.112	0.124	0.000
	AV	0.034	0.132	0.116	0.019	0.017	0.218	0.279	0.000	0.008	0.177	0.247	0.000	0.060	0.151	0.340	0.000
	Rep	0.000	0.051	0.040	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2016-17	SP	0.000	0.079	0.155	0.124	0.000	0.058	0.110	0.065	0.000	0.068	0.236	0.000	0.000	0.089	0.248	0.000
	JV	0.190	0.019	0.006	0.000	0.091	0.034	0.054	0.000	0.118	0.028	0.026	0.000	0.135	0.041	0.031	0.000
	AV	0.167	0.062	0.072	0.002	0.142	0.073	0.309	0.000	0.186	0.076	0.262	0.000	0.203	0.076	0.177	0.000
	Rep	0.000	0.055	0.071	0.000	0.000	0.014	0.051	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
2017-18	SP	0.000	0.039	0.076	0.178	0.000	0.055	0.070	0.112	0.000	0.020	0.110	0.175	0.000	0.008	0.044	0.118
	JV	0.124	0.013	0.015	0.000	0.169	0.044	0.019	0.000	0.070	0.029	0.030	0.000	0.110	0.048	0.033	0.000
	AV	0.170	0.068	0.103	0.018	0.068	0.097	0.238	0.008	0.235	0.053	0.103	0.000	0.060	0.120	0.343	0.000
	Rep	0.000	0.032	0.164	0.000	0.000	0.036	0.083	0.000	0.000	0.026	0.148	0.000	0.000	0.015	0.103	0.000

C. Appendix Figures

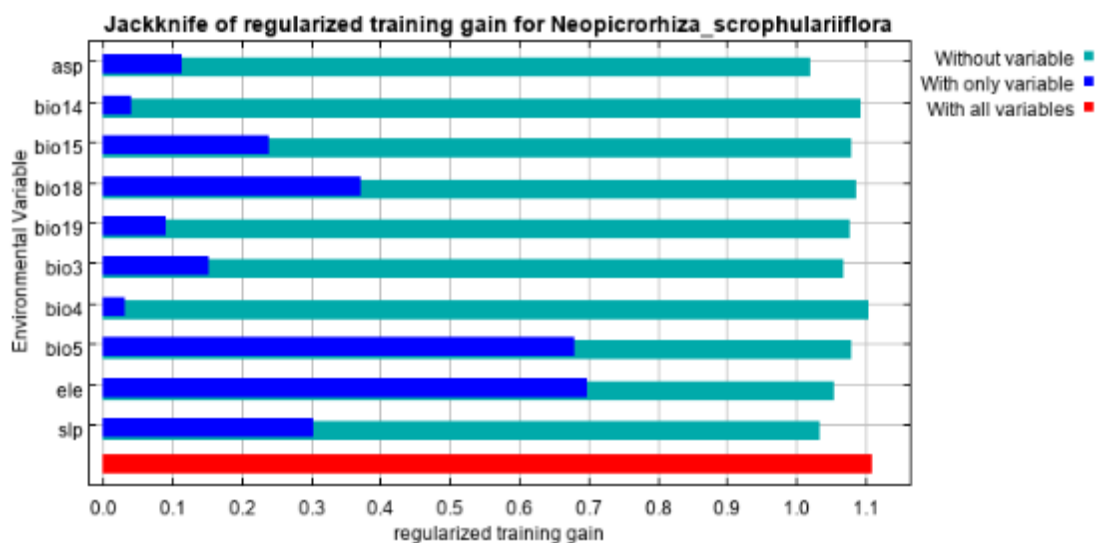


Figure C.1 *Neopicrorhiza scrophulariiflora* training data from the Himalaya Nepal using a Jackknife of mean regularized training gains for each variable over 50 trials. Table B.1 includes variable details

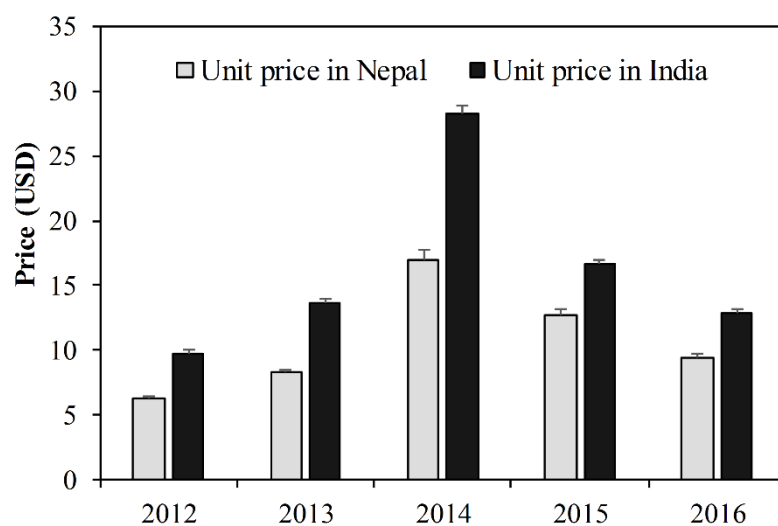


Figure C.2. *Neopicrorhiza scrophulariiflora* rhizome prices per kilogram in Nepal and India from 2012 to 2016 (Data source: ANSAB, <http://www.ansab.org/market-information/price-lists/>)

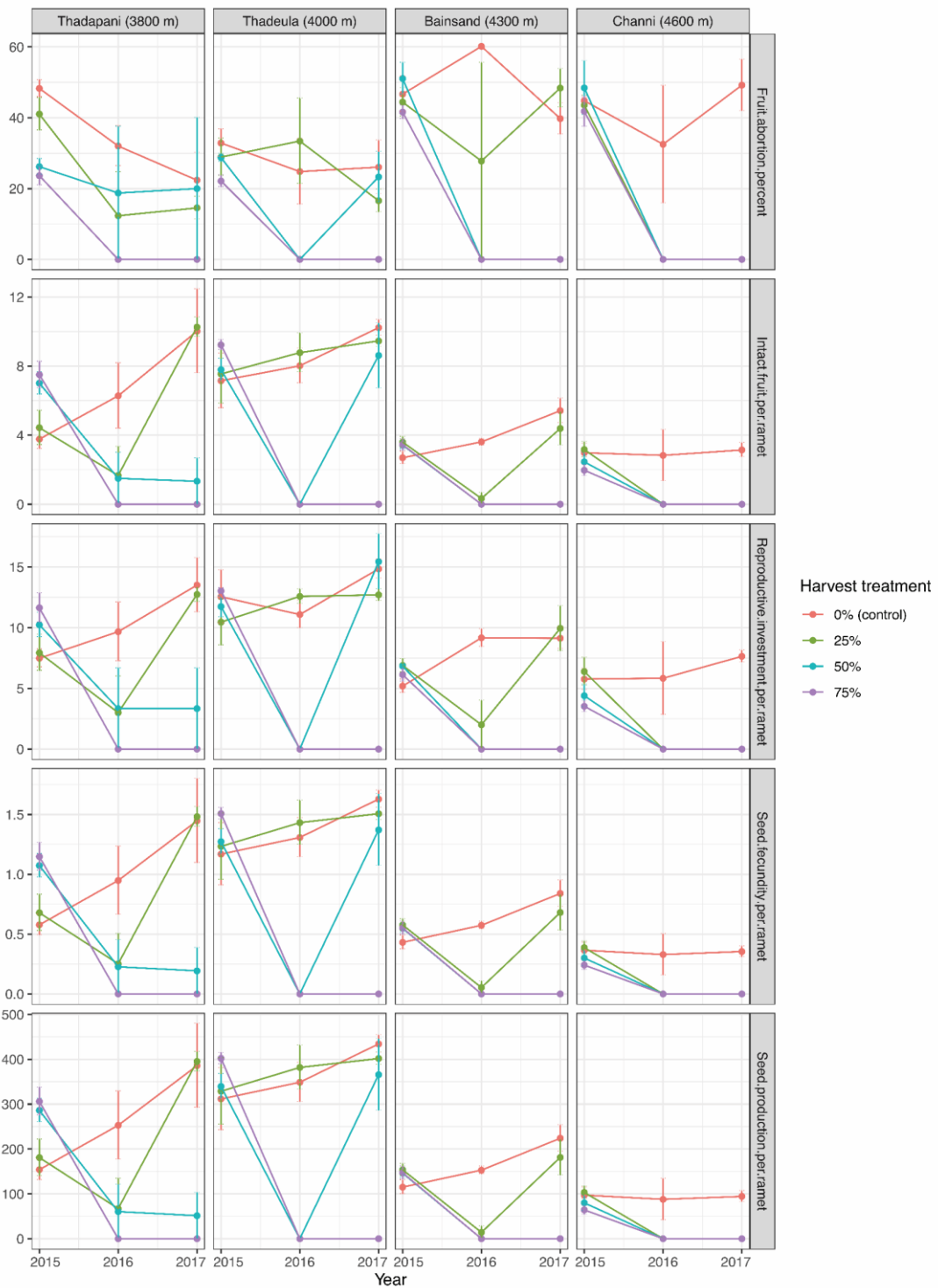


Figure C.3 Mean (\pm SE) reproductive activities per ramet of *Neopicrorhiza scrophulariiflora* from pretreatment of experimental harvest on density (m^{-2}) applied in the same year (2015) to two-years of postharvest-restoration period along the populations at Api-Nampa Conservation Area, in north-western Himalaya Nepal. Reproductive traits are presented sequentially top to bottom: fruit abortion percent, setting of intact fruit, reproductive investment (totaling all count values: flower + bud + aborted/intact fruit), potential seed fecundity (seed number \times proportion of seedling established from seed), and seeds set.

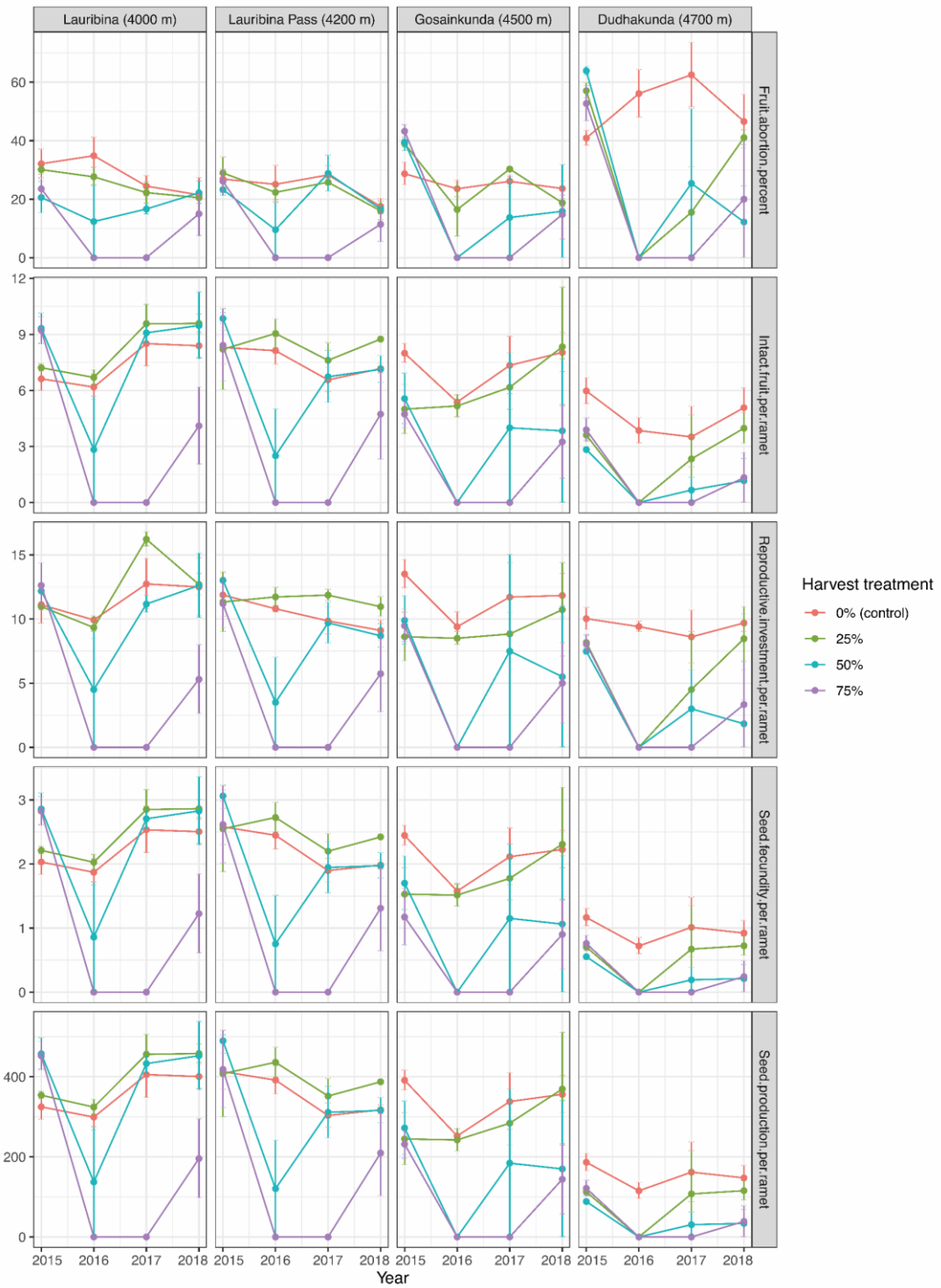


Figure C.4 Mean (\pm SE) reproductive activities per ramet of *Neopicrorhiza scrophulariiflora* from pretreatment of experimental harvest on ramet density (m^{-2}) applied in the same year (2015) to three-years of postharvest-restoration period along the populations at Langtang National Park, in north-central Himalaya Nepal. Reproductive traits are presented sequentially top to bottom: fruit abortion percent, setting of intact fruit, reproductive investment (totaling all count values: flower + bud + aborted/intact fruit), potential seed fecundity (seed number \times proportion of seedling established from seed), and seeds set.

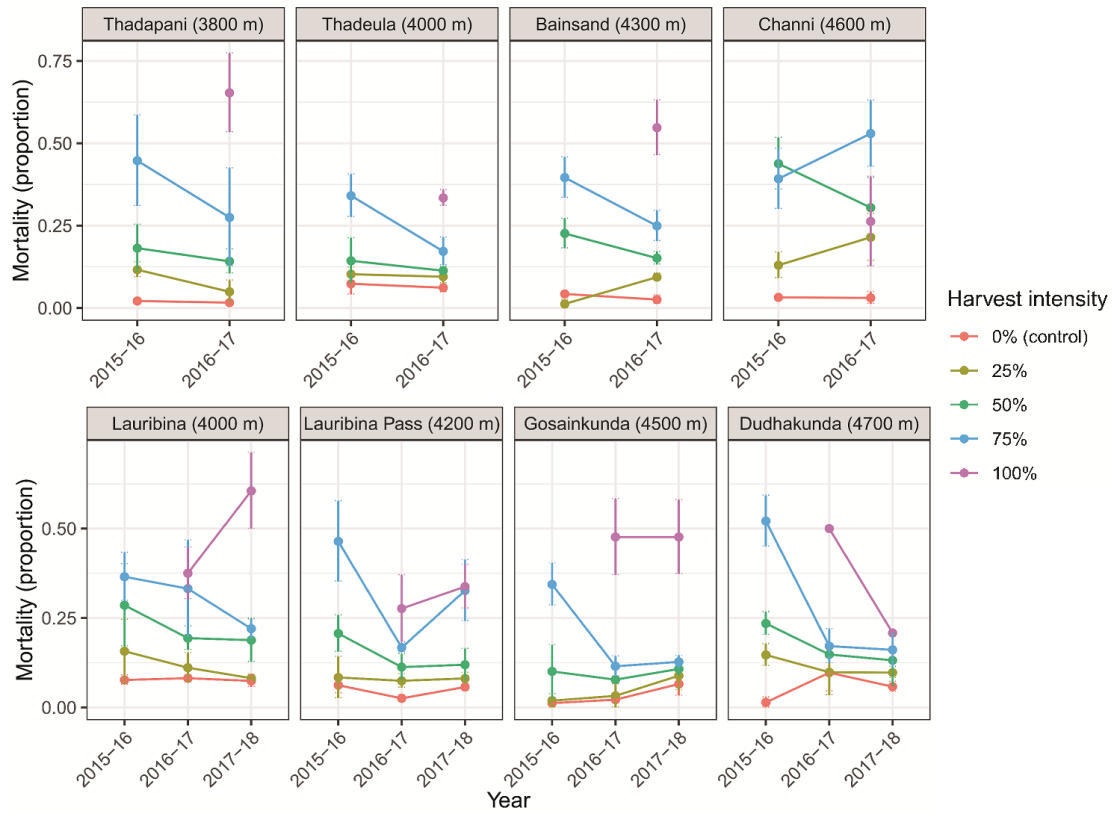


Figure C.5 Ratio (mean \pm SE) of ramet mortality (m^{-2}) at pretreatment level prior to experimental harvest treatment on density made in 2015 to a relative annual measure of postharvest periods of *Neopicrorhiza scrophulariiflora* along with populations in Api-Nampa Conservation Area (top panel, censused up to 2017), and Langtang National Park (bottom panel, censused up to 2018), in the Nepalese Himalaya.

D. Conferences Participated with Oral Presentation

D.1 International conference on Dynamic Borderlands: Livelihoods, Communities and Flows, 5th Conference of the Asian Borderlands Research Network by Social Science Baha, Kathmandu in 12–14 December, 2016, Title: Variation in life history traits of a commercially threatened medicinal herb, *Neopicrorhiza scophulariiflora*, along an environmental gradient in alpine Himalaya, Nepal

D.2 International conference on Wild Harvests, Governance and Livelihoods in Asia, organized by Transiting Green Growth Nepal and Science and Power in Participatory Forestry, Kathmandu in 30 November–02 December, 2017, Title: Effects of environmental factors along a harvest gradient on density and biomass of a clonal medicinal herb, *Neopicrorhiza scrophulariiflora* in alpine Himalaya, Nepal.

D.3 International conference on Biodiversity, Climate Change Assessment and Impacts on Livelihood organized by Central Department of Botany, Tribhuvan University, Kathmandu in 10 – 12 January, 2017, Title: Rarity and commonness in Himalayan Poppy (*Meconopsis* spp.): Variation in life history traits along an elevation gradient in alpine Himalaya, Central Nepal

D.4 International conference on Mountain in the changing world was organized by Kathmandu Institute of applied sciences in October 1-2, 2016, Title: Variation in life-history traits of the narrow endemic *Meconopsis napaulensis* (Papaveraceae) and its widespread congener *Meconopsis paniculata* along an elevation gradient in alpine Himalaya

D.5 Second Graduate Conference on Environment and Sustainable Development organized by Tribhuvan University, ISET Nepal and Resources Himalaya Foundation, Kathmandu, Nepal, 4-5 April 2016, Title: Rarity and commonness in Himalayan Poppies: Pollen attributes, seed output and germination traits variation in *Meconopsis napaulensis* and *M. paniculata*

D.6 Seventh National Conference, organized by Nepal Academy of Science and Technology (NAST), Kathmandu, Nepal, 29-31 March 2016, Title: Population growth performance in endemic Himalayan poppy *Meconopsis napaulensis* along an altitudinal gradient in central Nepal

E. Courses and Trainings Attended During PhD

S.N.	Course name	Participated date	Provided institution	ECTS
1	Biodiversity and Environmental Management	Nov. 2015–May 2016	Tribhuvan university	15
2	Introduction to new PhD students	25–31 March 2017	University of Copenhagen	3
3	Art of scientific writing	Feb.–June 2017	University of Copenhagen	3
4	Introduction to MATLAB for multivariate analysis	05–11 May 2017	University of Copenhagen	3
5	Training workshop on <i>Scientific Literature Review, Research Methodology and Academic Writing</i>	24–26 Nov. 2019	Tribhuvan University	
6	Training program on ' <i>Research Methodology for PhD Scholars</i> '	20-26 June 2017	Tribhuvan University	

F. Peer Review Work for Journal's Article Manuscript

F.1 *Agriculture, Ecosystems & Environment* [No. of manuscript reviewed: 2, Year: 2019], Publisher: Elsevier, Netherlands

F.2 *Ecological Informatics* [No. of manuscript reviewed: 1, Year: 2021], Publisher: Elsevier, Netherlands

F.3 *Ecological Processes* [No. of manuscript reviewed: 1, Year: 2021], Publisher: Springer Nature, Germany

F.4 *Modeling Earth System and Environment* [No. of article manuscript reviewed: 1, Year: 2021], Publisher: Springer Nature, Germany

F.5 *Journal of Applied Research on Medicinal and Aromatic Plants* [No. of manuscript reviewed: 1, Year: 2023], Publisher: Elsevier, Netherlands

G. Publication Profile

G.1 Research Papers From the PhD Dissertation

- **Poudeyal, M.R.**, Meilby, H., and Ghimire, S.K. Overharvesting is an issue, not harvest itself for commercially threatened *Neopicrorhiza scrophulariiflora* in Himalaya Nepal (Manuscript Prepared)
- **Poudeyal, M.R.**, Meilby, H., Hart, R., and Ghimire, S.K. (2024). Sustainable harvest of a threatened medicinal herb: Empirical evidence for spatially and temporally specific management of *Neopicrorhiza scrophulariiflora*. Submitted in: *Perspectives in Plant Ecology, Evolution and Systematics*64: 125799.
- **Poudeyal, M.R.**, Pyakurel, D., Rana, S.K., Meilby, H., Paneru, Y.R., Ghimire, S.K. (2021). Does resource availability coincide with exploitation patterns? Inference from distribution and trade of *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong in the Nepalese Himalaya. *Journal of Applied Research on medicinal and Aromatic Plants*, 22:100292.
- **Poudeyal, M.R.**, Meilby, H., Shrestha, B.B., Ghimire, S.K. (2019). Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas. *Ecology and Evolution*: 9(13): 7726–7740.

G.2 Co-authored and Additional Research Article Papers

- Nepal, S., Paudel, A., **Poudeyal, M.R.**, Paudel B.R., Jnawali, B., Ren, Z.-X., Wang H., Ghimire, S.K., Shrestha, M. Pollen ovule attributes along the elevational gradient in Nepalese Himalayas. *Oikos* (under review)
- Ghimire, N.P., Gurung A., K.C., Kushal, Paudel M.R., Bhattarai H.D., **Poudeyal, M.R.**, Ghimire S.K. (2023). Traditional socio-cultural utilization and harvesting practices of an alpine medicinal herb, *Neopicrorhiza scrophulariiflora* in Khumbu Valley, Nepal. *Ethnobotany Research and Applications*, 26:64.
- **Poudeyal, M.R.**, Ghimire, S.K. (2011) Habitat differentiation and population traits variation between the rare *Meconopsis napaulensis* and the common congener *M. paniculata*: implications for rare plant management. *Botanica Orientalis – Journal of Plant Science*, 8: 57–69.

G.3 Book and Book Chapters

- **Poudeyal M.R.**, Saud K, Paudel MR, Singh UK. 2024. The Significance and Utilization of *Pholidota* Orchids in Medicinal Preparations. In: Exploring Medicinal Orchids, Pant et al. (Eds.), CRC Press, USA
- Paudel, M.R., **Poudeyal, M.R.**, Devkota, H.P. (2022). *Ziziphus jujuba* Mill. In: Belwal, T., Bhatta, I.D., Devkota, H.P. (Eds), Himalayan Fruits and berries: Bioactive compounds, uses and nutraceutical potential. Elsevier, Netherlands (Pp. 491-496).
- **Poudeyal, M.R.**, Kunwar, R.M., Bussmann, R.W., Paniagua-Zambrana, N.Y. (2021). *Meconopsis aculeata* Royle, *Meconopsis horridula* Hook. f. and Thomson, *Meconopsis latifolia* (Prain) Prain, *Meconopsis quintuplinervia* Regel, *Meconopsis simplicifolia* (D. Don) Walpers, Papaveraceae. In: Kunwar, RM, Sher, H., Bussamann, R.W. (Eds), Ethnobotany of the Himalayas. Springer, USA.
- Ghimire S.K., Subedi C.K., Budha-Magar S., Adhikari M., Pandey T.R., Awasthi B., ThapaMagar S., **Poudeyal M.R.**, Ghimire K.M., Shrestha B.B., Bhatt G.D., Joshi L.R., Paudel A., Chapagain D.J. and Gurung J. (2021). Flora of Kailash Sacred Landscape Nepal: An Annotated Checklist. Volume 1 (Gymnosperms and Angiosperms: Ephedraceae – Buxaceae). Research Centre for Applied Science and Technology (RECAST), Tribhuvan University, Kathmandu, Nepal.
- Smith-Hall, C., Pouliot, M., Pyakurel, D., Fold, N., Chapagain, A., Ghimire, S.K, Meilby, H., Kmoch, L., Chapagain, D.J., Das, A., Jun, H., Nepal, K., **Poudeyal, M.R.**, Kafle, G., Larsen, H.O. (2018). Data Collection Instruments and Procedures for Investigating National-Level Trade in Medicinal and Aromatic Plants: The Case of Nepal. Department of Food and Resource Economics (IFRO), University of Copenhagen, Denmark, Documentation, No. 2018/2

G.4 Popular Articles in Local Journals

- **Poudeyal, M.R.** (2016). लोपउन्मुख औषधीजन्य वनस्पती हिमाली पोप्पी (*Meconopsis*): वातावरणीय प्रभाव र संरक्षण चूनौतीहरू (Rare Himalayan Plant

Poppy (Meconopsis): Environmental effect and conservation challenges). हाम्रो सम्पदा (Hamro Sampada), 16 (7): 46-50

- **Poudeyal, M.R.**, Thapa, H. (2013). औषधीजन्य वनस्पति: संरक्षण, सम्भावना तथा चुनौतीहरू (Medicinal Plants: Conservation, Possibilities and Challenges). हाम्रो सम्पदा (Hamro Sampada), 12 (10): 44-45
- Thapa, L.B., **Poudeyal, M.R.** (2013). सुदुर पश्चिम, सजिवन र सहकारी (Far Western, *Jatropha* and Co-operative). हाम्रो सम्पदा (*Hamro Sampada*), 12 (10): 37-39
- **Poudeyal, M.R.**, Ghimire, S.K. (2013). दुर्लभ-रैथाने वनस्पति, वातावरणीय प्रभाव र संरक्षणका चुनौतीहरू (Rare-endemic Plants: Ecological Impacts and Conservation Challenges). वैज्ञानिक जगत (Vaigyanik Jagat), 12 (12): 21-25

H. Permission Letter Working for the Protected Areas

	<p>नेपाल सरकार वन तथा जल-संरक्षण विभाग</p> <p>राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग (संरक्षण)</p>	<p>फोन नं. : ९७७-०१-४२५२००० ४२५२००१ ४२५२००२ फ्याक्स नं. ४२५२००३</p> 
<p>संकेत नं. : २०७३/३३ इकाई १५९ पत्र संख्या : १२० आवक नं. :</p>		<p>फोन नं. : ०१-४२५२००० ४२५२००१, ४२५२००२ E-mail: info@nepal.gov.np http://www.nepal.gov.np</p> <p>मिति: २०७३/०९/०८</p>

विषय:- अर्घखण अनुसन्धान अनुमति सम्बन्धमा ।
 श्री आंगटान राष्ट्रिय निकुञ्ज कार्यालय, मुग्लि, रतुवा ।
 श्री के.सि.सम्पदा (पिछला इन्डिया कार्यालय, एजिजेन, फ्रान्स) ।
 प्रस्तुत विषयमा त्वम संश्लिष्ट शोधमा विद्यमानुसार अर्घखण अनुसन्धान अनुमति प्रदान गरिएको स्वर्गीय आदेशानुसार अनुमति छ ।

अनुसन्धानकर्ताको नाम	पहिरो मुक्ति	बीचको नाम	धा पीडुवाल
अनुसन्धानकर्ताको ठेगाना	स्थानी: दिनाम-२, मुग्लि अस्थायी: किरिँट्ट, काठमाडौं	ई-मेल: rniktpoudyal@gmail.com	फोन नं.: ९८४१०५६७३१२
सम्बद्ध संस्था	संस्थाको नाम: विद्यमान विश्वविद्यालय, तनस्पाति शास्त्र केन्द्रीय विभाग	ठेगाना: किरिँट्ट, काठमाडौं	
पद	विद्यार्थी		
अनुसन्धानको लक्ष्य	पि.एच.डी	संस्थागत:	
अनुसन्धानको विषय	Population Ecology and Harvesting Sustainability of Some Important medicinal and Aromatic plants in Alpine Himalaya, Nepal		
अनुसन्धानको विधि	Field Sampling and monitoring	समुदाय संकलन: गर्मी	समुदायको परीक्षण: नेपालमा गर्मी
अनुसन्धानको समयवधि	September 1, 2015 देखि September 1, 2017 सम्म		
अनुसन्धानका सर्तहरू	१. अनुसन्धानकर्ताले राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन २०२९ र विधिसार्वली २०३० तथा यस माहाकान्ता सभै विधिसार्वलिकको पूर्ण पालना गर्नु पर्ने छ । २. अनुसन्धानकर्ताले विभाग र सम्बन्धित संश्लिष्ट क्षेत्र कार्यलय सँग सम्बन्ध गरि कार्य गर्नु पर्ने छ ।		

	<p>नेपाल सरकार वन तथा जल-संरक्षण विभाग</p> <p>राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग (संरक्षण)</p>	<p>फोन नं. : ९७७-०१-४२५२००० ४२५२००१ ४२५२००२ फ्याक्स नं. ४२५२००३</p> 
<p>संकेत नं. : पत्र संख्या : आवक नं. :</p>		<p>फोन नं. : ०१-४२५२००० ४२५२००१, ४२५२००२ E-mail: info@nepal.gov.np http://www.nepal.gov.np</p>

	१. अनुसन्धानकर्ताले आफ्नो अनुसन्धानको प्रस्ताव सम्बन्धित संश्लिष्ट क्षेत्र कार्यालयमा सहीत पेश गर्नु पर्नेछ । २. अनुसन्धानकर्ताले अनुसन्धान सफल भएपछि एक प्रति कागजी प्रतिवेदन र एक प्रति विपुलीय प्रतिवेदन विभाग र सम्बन्धित संश्लिष्ट क्षेत्र कार्यालयमा बुझाउनुपर्ने छ । ३. अनुसन्धानकर्ताले भविष्यका प्रकृतित सदा अनुसन्धानमा संलग्न बर्ज्यादीको पौढाङको आयातमा सह लेखनको रूपमा समवेत गर्नुपर्नेछ । ४. संश्लिष्ट मापदण्ड विदेश गरीजने ।
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 श्री राजमराट
 सहायक इन्फोर्मेन्टिभ

शोधार्थी:

अनुसन्धानकर्ता श्री मुक्ति राज पीडुवाल :- सम्बन्धित संश्लिष्ट क्षेत्र कार्यालय सँग सम्बन्ध गरी अनुसन्धान अनुसन्धान गर्नु हुन।

I. Research Articles Published From Dissertation

Appendix I.1: Poudeyal, M.R., Pyakurel, D., Rana, S.K., Meilby, H., Paneru, Y.R., and Ghimire, S.K. (2020). Does resource availability coincide with exploitation patterns? Inference from distribution and trade of *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong in the Nepalese Himalaya. *Journal of Applied Research on medicinal and Aromatic Plants*, 22, 100292.

<https://doi.org/10.1016/j.jarmap.2021.100292> (Impact factor: **3.95**)

The screenshot shows the ScienceDirect article page. The browser address bar displays the URL: [sciencedirect.com/science/article/abs/pii/S2214786121000012](https://www.sciencedirect.com/science/article/abs/pii/S2214786121000012). The ScienceDirect logo is visible in the top left, and 'Journals & Books' is in the top right. Below the logo, there are navigation options: 'View PDF' and 'Access through your Institution'. The article title is 'Does resource availability coincide with exploitation patterns? Inference from distribution and trade of *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong in the Nepalese Himalayas'. The journal information is 'Journal of Applied Research on Medicinal and Aromatic Plants', Volume 22, April 2021, 100292. The authors listed are Mukti R. Poudeyal, Divesh Pyakurel, Santosh K. Rana, Henrik Meilby, Yagya R. Paneru, and Suresh K. Ghimire. The page includes a table of contents on the left with sections like Outline, Highlights, Abstract, Keywords, and Introduction. At the bottom, there are options to 'Add to Mendeley', 'Share', and 'Cite'.

Appendix I.2: Poudeyal, M.R., Meilby, H., Shrestha, B.B., and Ghimire, S.K. (2019). Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas. *Ecology and Evolution*: 9(13): 7726–7740, DOI: 10.1002/ece3.5355 (Impact factor: **2.6**)

The screenshot shows the Wiley Online Library page for the article. The browser address bar displays the DOI: 10.1002/ece3.5355. The journal title "Ecology and Evolution" is prominently displayed with an "Open Access" badge. The article title is "Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas". The authors listed are Mukti Ram Poudeyal, Henrik Meilby, Bharat Babu Shrestha, and Suresh Kumar Ghimire. The publication date is 19 June 2019. A "Data Availability Statement" is provided, stating that data used for ecological analysis are available, but human participant data is not. The abstract begins with "A surprisingly large number of species potentially threatened by human harvest lack quantitative ecological studies incorporating harvest effects, especially clonal species in the alpine Himalayas. We studied density and biomass variation of a threatened medicinal herb, *Neopicrorhiza scrophulariiflora*, to examine the effect of harvest on plant". On the right side, there is a cover image of the journal issue (Volume 9, Issue 13, July 2019, Pages 7726-7740) and an advertisement for the journal featuring a portrait of Marcus Lashley, the new co-editor-in-chief. At the bottom right, there is a "Citation Statements" section showing 1 supporting, 1 mentioning, and 0 contrasting citations.



Does resource availability coincide with exploitation patterns? Inference from distribution and trade of *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong in the Nepalese Himalayas

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ARTICLE INFO

Keywords:

Medicinal plants
Maximum entropy
Habitat suitability
Traded volume
Economic contribution
Sustainable conservation

ABSTRACT

Despite the widespread use and trade of the highly valued Himalayan medicinal plant, *Neopicrorhiza scrophulariiflora*, there is still inadequate information on its distribution and on the environmental factors that define suitable habitats. Such information is needed to identify suitable areas for sustainable resource extraction. We hypothesize that there is a discrepancy between the geographical distribution of trade and climatically suitable areas and suggest that this could lead to a risk of local depletion of the species, which could seriously affect rural livelihoods. To address this hypothesis, we conducted species distribution modeling of *N. scrophulariiflora* using Maximum Entropy with ten environmental variables and 63 species occurrence records (after rarefaction) from Nepal and related the resulting distribution model to trade assessment statistics from 12 fiscal years (2004–2016). The predicted area of suitable habitat in Nepal was estimated at 11,617 km², and highly suitable areas were located in a narrow elevational range (4000–4400 m), with a predicted area of 386 km² (0.3 %). Suitable and highly suitable areas were mostly located in the eastern mountains, probably due to mild temperatures and adequate precipitation around the peak of the plants' growing season. Available official trade records indicated that Nepal exported only 372 tons of *N. scrophulariiflora* rhizomes in 2004–2016 (average: 31 tons/annum), mostly from the western mountains where predicted habitats were classified as less suitable. Discordant geographical patterns of habitat suitability, extraction from habitats of low suitability, and increasing trade indicate that the current resource exploitation is likely to be unsustainable. We suggest formulating management strategies for locations with heavy collection and trade, conducting cultivation trials in suitable patches, and identifying sustainable annual harvest limits.

1. Introduction

Medicinal plants in mountain ecosystems are an important basis for traditional health care systems and offer an opportunity to generate cash income in rural areas of developing countries (Hamilton, 2004; Jensen and Meilby, 2008). However, a significant number of alpine medicinal plants are confined to narrow habitat ranges and are vulnerable to environmental change, habitat loss or modification, and destructive commercial harvest (Hamilton, 2004; Cunningham et al., 2018; Applequist et al., 2020). As a result, to conserve such species, it is important to

maintain the stability of ecologically important habitats, and an urgent research effort is needed to evaluate the risk of extinction. To do this, it is necessary to understand the species' ecological niches, examine their spatial distributions, assess potential threats, prioritize habitat areas for conservation, and to restore threatened ecosystems (Manish and Pandit, 2019; Rana et al., 2020). Globally, species conservation and management decisions are largely based on known sites of occurrence, either recorded through direct field observations or indirectly based on specimens deposited in museums and herbaria (Phillips and Dudik, 2008; Rana et al., 2020; Nagahama and Bonino, 2020). Estimation of the

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potential area of distribution and suitable habitats of mountain plants is imperative to assess their vulnerability to changing environmental conditions, whether caused by natural or anthropogenic factors. Species Distribution Models (SDM) based on climatic and topographic variables could be useful to obtain estimates of spatial distribution, potential abundance, and environmental adaptability, to find appropriate harvesting locations, and to develop effective plans for sustainable conservation (Shrestha and Bawa, 2014; Nagahama and Bonino, 2020). Moreover, SDM is considered as a cost-effective tool for providing information on ecological characteristics, factors shaping populations and connectivity, and habitat requirements within a relatively short time (Poudel et al., 2012; Sharma et al., 2018).

'Kutki' is a trade name used for four different species of commercial medicinal plants, globally recognized as belonging to two Himalayan genera: *Neopicrorhiza* [*N. scrophulariiflora* (Pennell) D.Y. Hong and *N. minima* R.R. Mill)] and *Picrorhiza* [*P. kurrooa* Royle ex Benth. and *P. tungnathii* Pusalkar] (Hong, 1998; Pusalkar, 2014). Rhizomes of all four species are used in traditional medicine. All four species have localized distributions in a narrow band of subalpine–alpine areas of India, Nepal, Bhutan, Tibet, and western China at 2700–5000 m above sea level. Among the four species, *P. kurrooa* and *N. scrophulariiflora* have been traded for millennia, thereby offering an opportunity to generate additional income for rural households in the Himalayas (Olsen, 2005; Uniyal et al., 2011; Sharma and Kala, 2018). However, possibly due to the inclusion of *P. kurrooa* in CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora) Appendix II in 1997, the trade pressure has shifted towards *N. scrophulariiflora* (Mulliken, 2000). In Nepal, Kutki is represented by a single species, *N. scrophulariiflora* (Smit, 2000; Olsen, 2005; Ghimire et al., 2005; Shrestha and Jha, 2009; Poudyal et al., 2019), which is considered as highly threatened due to excessive commercial harvesting, presumably beyond its regeneration capacity (Bhattarai et al., 2002; Ghimire et al., 2005). The traditional system of harvesting, which involves low intensity extraction of rhizomes for subsistence use at intervals, limits the local impact on populations (Ghimire et al., 2005), but overharvesting to meet the high demand of international markets seems problematic, even at the local level (Poudyal et al., 2019).

The annual supply of 'Kutki' rhizomes from Nepal, Bhutan, and India to the global market was estimated at 650–1000 tons, 50–300 tons of which was contributed by *P. kurrooa* and the rest by *N. scrophulariiflora* (Olsen, 2005; Uniyal et al., 2011). Nepal is the largest global supplier of Kutki rhizomes; the production amounts to 175–770 tons per annum, which is about 66 % of the global production (Olsen, 2005). Recently, Pyakurel et al. (2018) reported that the export of high-value medicinal plants from the western Himalayas of Nepal has increased two-fold by volume and 17 times by value within the last two decades. The trade network is characterized by low transparency, only little information is available on harvesting and trade, and no comprehensive biophysical studies are conducted before issuing harvest permits (Updret et al., 2016; Devkota et al., 2017; Pyakurel et al., 2018). Therefore, to increase the chances of maintaining supplies and achieving sustainable management in the future, reliable information on the species' occurrence and extraction potentials are urgently needed.

Despite its importance in traditional and modern medicines, and increasing traded volumes and rising prices, the distribution and habitat requirements of *N. scrophulariiflora* have not been sufficiently studied. However, previous studies have reported that the distribution is affected by species demographic constraints, harvest, habitat exploitation and destruction, and changing climatic conditions (Ghimire et al., 2005; Rana et al., 2017; Poudyal et al., 2019; Sharma and Kala, 2018; Applequist et al., 2020). In this study, we attempt to increase our knowledge by identifying factors that affect habitat suitability, estimating the distribution and area of suitable habitat, and by examining the trade of Kutki from the Nepalese Himalayas to elucidate current problems and to inform sustainable management. We hypothesize that there is a discrepancy between the geographical distribution of trade

and climatically suitable areas and suggest that this could lead to a risk of local depletion of the species, which could seriously affect rural livelihoods and long-term management. Identifying areas with optimal habitats and suggesting production alternatives (e.g., cultivation) may counteract over-exploitation and supplement the resource. We sought to answer the following questions: (1) What are the potential areas of suitable (high, medium, and low) and unsuitable habitats for *N. scrophulariiflora* within its natural range in Nepal? Does habitat suitability vary across geographical regions? (2) What are the main ecological determinants that can be used to quantify the occurrence of the species within its entire range in the Nepalese Himalayas? (3) What are the current trade patterns like in Nepal? (4) Does the trade align with the estimated distribution pattern in the Nepalese Himalayas? To answer these questions, we evaluated habitat suitability based on recorded occurrences of *N. scrophulariiflora* populations and used suitable bio-climatic variables to classify potential habitat areas across Nepal. Next, we examined available trade data and compared the geographical distribution of habitat suitability classes with the information on trade patterns and revenue collection. This way we provide novel insights regarding the trade-habitat interplay to address sustainability issues and support the conservation of medicinal plants.

2. Material and methods

2.1. Study species

Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong (Synonym: *Picrorhiza scrophulariiflora* Pennell, Family: Plantaginaceae) is a rhizomatous perennial herb with fragmented distribution at 3500–4800 m a.s.l. in the Nepalese Himalayas. The plant bears multiple dark blue flowers in a long raceme starting from June, borne on a stout stem arising from a rosette of leaves. Fruit maturation and seed dispersal take place in September and October. Growth is slow, sexual reproduction is often rare, and expansion and colonization take place by vegetative shoots which help to develop clumpy patches (Ghimire et al., 2005). Shrubland meadows dominated by *Rhododendron* species provide favorable conditions for *N. scrophulariiflora* populations (Ghimire et al., 2005; Poudyal et al., 2019). The species has not been assessed and assigned a global conservation category, but at the national level, it was enlisted as 'vulnerable' by the Conservation Assessment and Management Plan (CAMP 2001) workshop (Bhattarai et al., 2002) and has been banned for export without identification and certification by the Government of Nepal (HMG/MoFSC, 2001).

As a consequence of growing demand the competition among collectors is increasing and the commercial collection now mostly takes place before seed maturation, from August to October, thereby threatening the stability of wild populations (Ghimire et al., 2005; Poudyal et al., 2019). Domestication of medicinal and aromatic plants (MAPs) is considered as an alternative to wild harvest that may help reducing the overexploitation, and trial cultivation of *N. scrophulariiflora* has been attempted in different parts of the Himalayas. However, inadequate understanding of the roles of climatic (temperature, precipitation), topographic (slope, aspect), and edaphic (soil nutrient) factors as determinants of habitat suitability have limited the success of the cultivation trials (Chandra et al., 2006; Cui et al., 2012).

Rhizomes of *N. scrophulariiflora* are used in Ayurvedic and Tibetan medicines as well as in folk healing systems to treat a variety of ailments, including cold, fever, asthma, jaundice, malaria, high blood pressure, intestinal pains, conjunctivitis, urinary disorders, and snake bites (Lama et al., 2001; Manandhar, 2002; Ghimire et al., 2005; DPR, 2006). Acknowledging some of its useful properties, modern pharmacology has also adopted the species (Smit, 2000; Liu et al., 2011; Uniyal et al., 2011; Kumar et al., 2017). Wider application of traditional medicinal practices and recent pharmacological evidence regarding the species further increases its market value. Owing to its pharmacological uses and the associated trade potential, the Government of Nepal has prioritized

N. scrophulariiflora for research and economic development, and for the development of agro-technological methods (DPR, 2006).

2.2. Study area

A herbarium survey and government statistics showed that *Neopicrorhiza scrophulariiflora* is found and traded in sub-alpine and alpine areas within 20 mountain districts from eastern to western Nepal (Fig. 1). Phyto-geographically, Nepal is divided into the western (80°04'–83°0' E), central (83°0'–86°30' E) and eastern (86°30'–88°12' E) regions (Stearn, 1960). The monsoon lasts longest and the precipitation is highest in the eastern region, particularly at elevations >2000 m, and decreases both in terms of duration and frequency towards the western region (Miehe et al., 2001; Böhner et al., 2015). The large altitudinal variation within short distances (below 100 m in the southern lowlands to the 8848.86 m of Mount Everest within an average distance of 193 km), together with a large variation in annual rainfall and temperature, are the basis for a large diversity of habitats, and the country is home to a large number of medicinal plant species. Out of 1950–2331 species of MAPs reported from Nepal (Ghimire, 2008; Rokaya et al., 2012), a total of 300 species are harvested for trade (Pyakurel et al., 2019). Twenty species of commercial MAPs, including *N. scrophulariiflora*, cover almost 80 % of the total traded volume (Heinen and Shrestha-Acharya, 2011), but the geographical distributions of the species have not yet been mapped or fully understood (but see Poudel et al., 2012; Rana et al., 2020; Kunwar et al., 2020).

2.3. Species location data

Neopicrorhiza scrophulariiflora occurrence records were collected from direct field observations made by the authors; herbarium specimens deposited at the National Herbarium and Plant Laboratory (KATH) and Tribhuvan University Central Herbarium (TUCH), Nepal; online databases of Tokyo University Herbarium (TI; <http://umdb.um.u-tokyo.ac.jp/DShokubu/>), Royal Botanical Garden Edinburgh Herbarium (E; <http://data.rbge.org.uk/search/herbarium/>), Flora of Nepal (<http://www.floraofnepal.org/data>), Global Biodiversity Information

Facility (GBIF; <https://www.gbif.org/>), and observations reported in published and unpublished literature. Most of the field observations were made by one of the co-authors (S.K. Ghimire) during field trips in 1995–2019. Additional geographical coordinates of *N. scrophulariiflora* populations were obtained from Lama et al. (2001), Manandhar (2002), Ghimire et al. (2005), Ghimire et al. (2008), Ghimire (2009), Shrestha and Jha (2009), and Poudeyal et al. (2019). Altogether, we collected ~250 occurrence points covering locations across the entire range within Nepal. After the removal of duplicates, we ended up with a shortened list of 84 localities without duplication.

To reduce spatial correlation in the dataset, we used a spatial rarefaction tool in the SDMToolbox (version: 2.4) in ArcGIS, version 10.5 (Rana et al., 2017, 2020). A minimum filter distance of 10 km is considered appropriate in areas with high spatial heterogeneity, such as mountainous regions (Rana et al., 2020). Therefore, the 84 short-listed locations were rarefied using a minimum distance of 10 km between neighboring locations to reduce the likely effects of spatial autocorrelation and the possible bias caused by over-sampled regions. This way we finally extracted 63 records, which we used to calibrate an SDM to identify relationships between the occurrence of the species and a set of environmental variables.

2.4. Environmental predictor variables

Nineteen bioclimatic variables were derived from a global interpolated climate dataset (<https://worldclim.org/version2>; Fick and Hijmans, 2017) and used as potential predictor variables. In addition, since topographic variables are often believed to influence the distribution of plant populations (Shrestha and Jha, 2009; Poudeyal et al., 2019), we included three different topographic variables (elevation, aspect, and slope) as potential predictors of the distribution of *N. scrophulariiflora*. The bioclimatic variables were derived from average monthly climate data (maximum and minimum temperature, and total precipitation) from a reference period 1970–2000, prepared using a global set of weather stations and interpolated at a resolution of 30 arc seconds, corresponding to grid cells with an area of approximately 1 km² (Fick and Hijmans, 2017). The nineteen bioclimatic variables represent

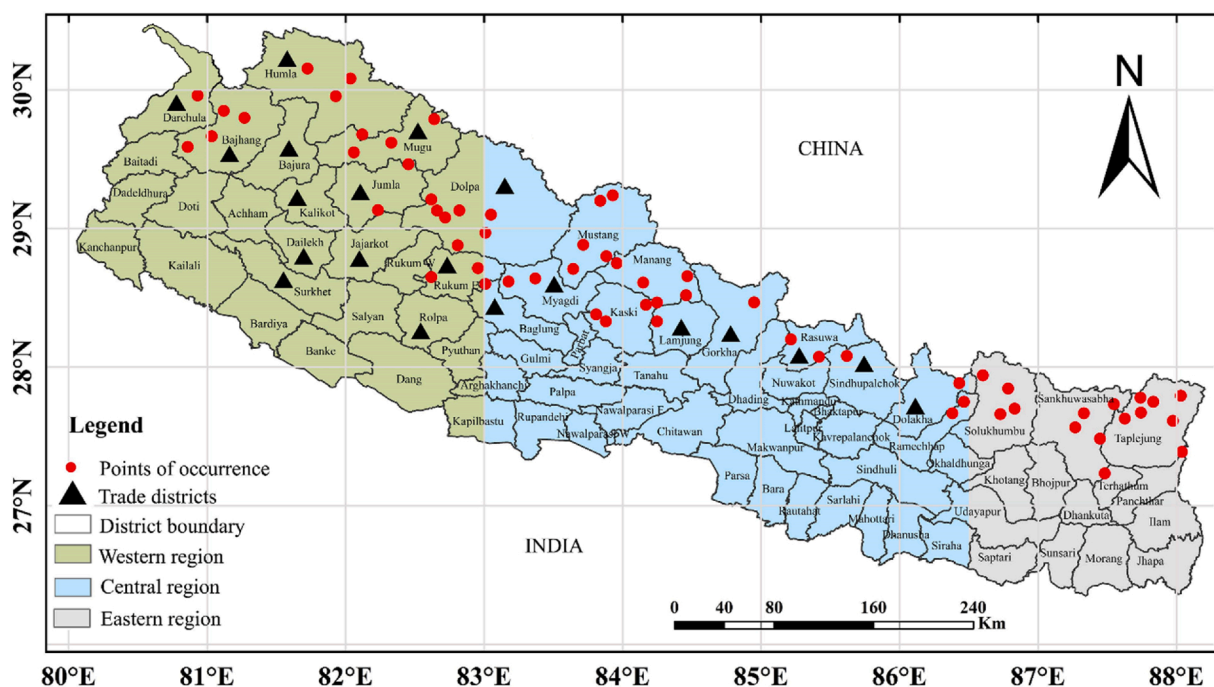


Fig. 1. Distribution map of *Neopicrorhiza scrophulariiflora* showing the origin of herbarium specimens (based on herbarium survey) and trade history records for 20 districts in the Nepalese Himalayas. Color zones indicate three ecological regions, western, central and eastern (adopted from Stearn (1960)).

annual variation, seasonality, and extreme or limiting temperature and precipitation, all of which are potentially relevant to describe the eco-physiological conditions that determine the occurrence of *N. scrophulariiflora* populations. To derive topographic variables (aspect and slope) at the appropriate resolution we used a Digital Elevation Model based on the Shuttle Radar Topographic Mission (SRTM) (provided by United States Geological Survey), which we resampled using the nearest neighbor resampling technique. All environmental variables were thus available at a spatial resolution of 30 arc seconds (~1 km² grid resolution) (Table A.1).

The collinearity among the bioclimatic variables is high within relatively small geographic areas (Rana et al., 2020). Using the 'car' package in the R-programming language (R Core Team, 2019; Table A.2) a variance inflation factor (VIF; Fox and Weisberg, 2011) was calculated with elevation as a response variable and used in combination with a Pearson correlation matrix to remove highly correlated variables. This was done to avoid multi-collinearity and over-parameterization of the model (Shrestha and Bawa, 2014; Table A.3). Hence, the final subset of predictor variables includes only variables with VIF values <10, and Pearson coefficients of correlation <0.8. Consequently, we ended up with a set of predictors including only seven bioclimatic and three topographic variables (Table 1).

2.5. Species distribution modeling

Model calibration based on presence-only data is best done using the Maximum Entropy (MaxEnt, version 3.4.1) approach to predict the potential distribution of a species (Phillips and Dudik, 2008). Provided a dataset with sufficient coverage, it gives robust estimates of habitat suitability even with limited data and for species characterized by a patchy distribution with a narrow spatial extent, such as for *N. scrophulariiflora* populations (Phillips and Dudik, 2008; Rana et al., 2020). To model the distribution of *N. scrophulariiflora*, MaxEnt used 25 % random test and 75 % training records from the original presence dataset with a single regularization multiplier against 10,000 randomly distributed background points. We calibrated the model using 50 replications with bootstrapping (Rana et al., 2017), the default convergence threshold value of 0.00001, and a maximum of 5000 iterations. Moreover, we used linear, quadratic, product, threshold and hinge features of the MaxEnt algorithm (Shrestha and Bawa, 2014).

The predictive power of the MaxEnt model was evaluated using TSS (True Skill Statistics), Cohen's Kappa, and AUC (Area Under Curve-Receiver Operating characteristics) statistics (Allouche et al., 2006;

Table 1

Predictor variables used to calibrate the species distribution model for *Neopicrorhiza scrophulariiflora* in the Nepalese Himalayas.

Environmental variable	Characteristic description
Geographical region	Nepal (Datum: WGS 1984)
Resolution	30 arc seconds
Climatic factor	Source: Worldclim (Fick and Hijmans 2017)
bio3	Isothermality (bio2/bio7) (* 100)
bio4	Temperature seasonality (standard deviation *100)
bio5	Maximum temperature of warmest month
bio14	Precipitation of driest month
bio15	Precipitation seasonality (coefficient of variation)
bio18	Precipitation of warmest quarter
bio19	Precipitation of coldest quarter
Topographic factor	Source: SRTM DEM (United States Geological Survey)
Elevation	Terrain elevation based on Digital Elevation Model (unit: meters)
Aspect	Aspect in degrees (cardinal direction, orientation of slope)
Slope	Slope in degrees (gradient for incline of surface)

Units: temperature in °C (degree Celsius), precipitation in mm (millimeter); SRTM DEM, Shuttle Radar Topographic Mission Digital Elevation Model.

Rana et al., 2019). TSS is independent of prevalence, i.e. the ratio of presence to pseudo-absence data in the presence-absence predictions (Allouche et al., 2006). This statistic deals with both sensitivity and specificity and its value ranges from -1 to +1, where +1 indicates perfect agreement, and values ranging from 0.7 to 0.9 specify fair to good model performance (Allouche et al., 2006). The independent area under curve (AUC) statistic describes the model's discriminatory ability and was calculated from the receiver operating characteristics (ROC) (Rana et al., 2017). When examining model performance, AUC metric values between 0.5–0.7 were considered as poor, 0.7–0.9 moderate, and >0.9 as good (Manel et al., 2001). In addition, we used response curves to assess which variables had significantly affected the probability of the presence of *N. scrophulariiflora* (Phillips and Dudik, 2008). The Boyce index was used to evaluate how well the model distinguishes between suitable and unsuitable areas (Manly et al., 2002). Boyce index (Spearman's correlation coefficient) values of at least 0.90 were considered to indicate that the model accurately defined suitable areas. The potential habitat distribution was mapped using four suitability classes; unsuitable (<25 % probability of presence), low (25–50 %), medium (50–75 %), and high suitability (>75 % probability of presence) (Shrestha and Bawa, 2014; Rana et al., 2019; Kunwar et al., 2020). We used equal training sensitivity and specificity logistic thresholds to delimit suitable areas from unsuitable areas (Pearson et al., 2007). Using a base map of Nepal and potential habitat predictions from our model, we prepared a map of currently suitable habitats of *N. scrophulariiflora*. This map was used in calculating the area of suitable habitats within three phytogeographic regions (Fig. 1), within protected areas, and within individual districts.

2.6. Trade information

Harvesters collect *Neopicrorhiza scrophulariiflora* from sub-alpine and alpine meadows and sell dried and cleaned products either to sub-local traders in villages, or to local traders at major road heads or district headquarters. Sub-local traders often receive a cash advance from local traders and never transport MAPs across district borders. Once the agreed volume (usually a tractor load) has been collected, local traders visit sub-local traders, settle advances and transport the products to their storehouse. Before this happens, the local trader receives a collection permit issued by the Divisional Forest Office (previously the District Forest Office) or the Conservation Area Office (in protected areas) and distributes copies to sub-local traders. Local traders receive a transport permit based on the collection permit, transport the products out of the district to a central wholesalers' storehouse (see details in Pyakurel et al., 2018; Smith-Hall et al., 2018). Finally, wholesalers receive a phytosanitary certificate, pay the customs tax, receive a transboundary export permit, and export the products to India, China, or other countries. Department of National Park and Wildlife Conservation (DNPWC), Government of Nepal, and local authorities (e.g., Conservation Area Offices) regulate the medicinal plants trade from protected areas, whereas Department of Forests (DoF) and their local Divisional Forest Offices regulate the trade outside the protected areas. These authorities record information on the quantity (volume) produced according to permits issued and the revenue collected.

For this study, we obtained DoF trade data from 12 fiscal years (2004/05 to 2015/16), which include information on the district of origin. We aggregated the district-level data to obtain estimates of national-level trade for each fiscal year. We correlated the suitable habitat area predicted by the SDM model with cumulative trade volume and annual trade frequency for the combined dataset including all districts to determine whether the existing exploitation pattern aligned with the predicted distribution of *N. scrophulariiflora*. The trade data from DoF, 2016DoF (2004–2016) included records of a range of traded medicinal plants (50–70 species per year). For each year we calculated percentages of total volume (quantity) and value (revenue) to determine the contribution of *N. scrophulariiflora* to the overall trade in medicinal

plants. We used price data from ANSAB (Asia Network for Sustainable Agriculture and Bioresources, <http://www.ansab.org/market-information/price-lists/>) to estimate market prices for *N. scrophulariiflora* in the five years from 2012 to 2016. We used price-lists for Kathmandu and Nepalgunj in Nepal for a national trend analysis, and for Delhi, Lucknow, Tanakpur and Kolkata in India for a regional trend analysis. The annual average price per kilogram was calculated as the average monthly price for each year and was multiplied by the average annual traded volume to estimate the annual trade value. We calculated prices

in US Dollars (USD) using the annual average exchange rate provided by Nepal Rastra Bank (<https://www.nrb.org.np/>).

3. Results

3.1. Importance of environmental factors

MaxEnt modeling successfully delineated the potential distribution of *Neopicrohiza scrophulariiflora* using seven climatic and three

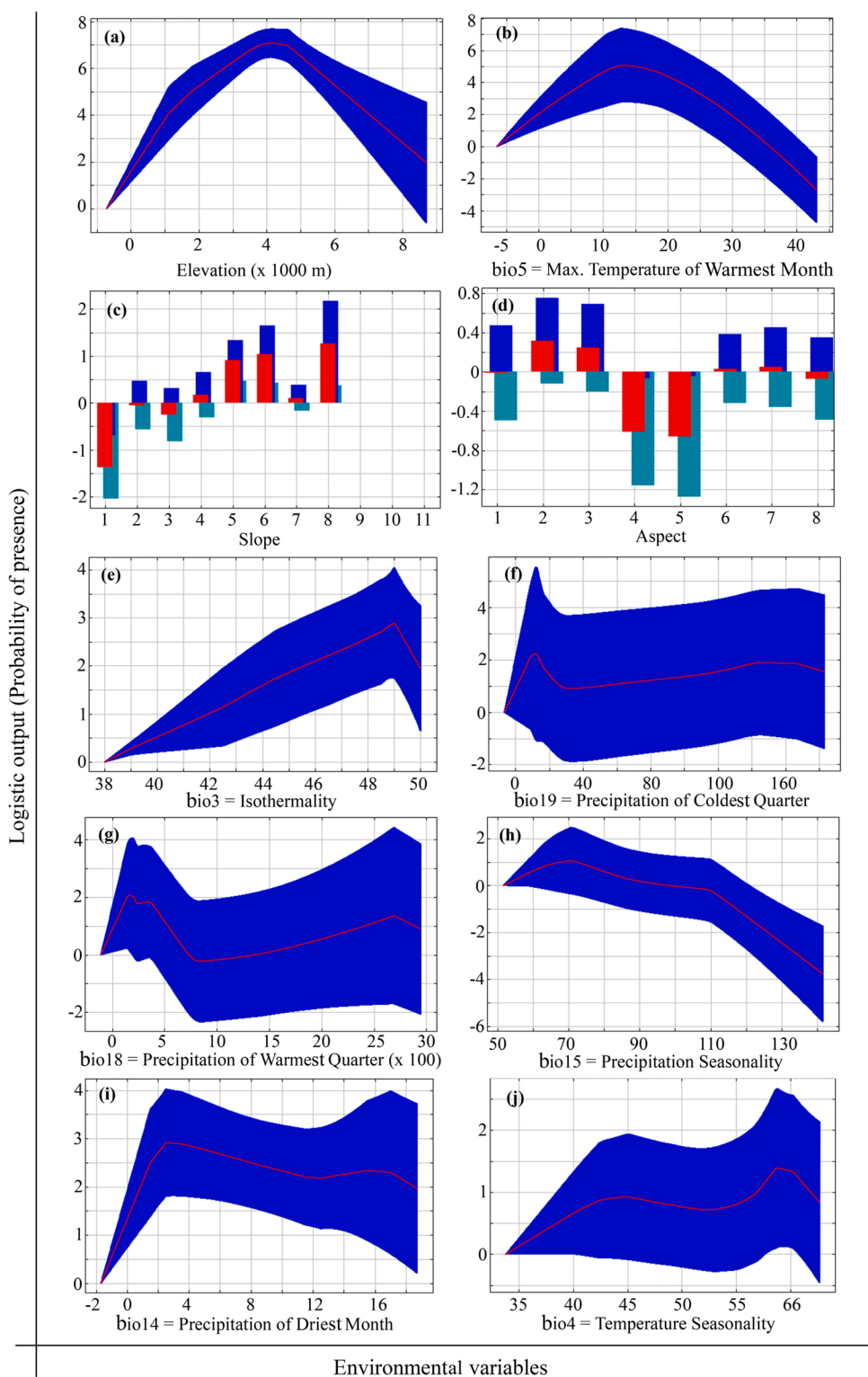


Fig. 2. Response curves for predictor variables used in modeling the probability of presence (logistic model) of *Neopicrohiza scrophulariiflora*. Panels show (a) Elevation, (b.) bio5 = Maximum temperature of warmest month, (c) Slope, (d) Aspect (e.) bio3 = Isothermality, and (f.) bio19 = Precipitation of coldest quarter, (g.) bio18 = Precipitation of warmest quarter, (h.) bio15 = Precipitation seasonality, (i.) bio14 = Temperature of driest month, and (j.) bio4 = Temperature seasonality. Temperatures are measured in °C (degree Celsius) and precipitation in mm (millimeters). Aspects are represented by 1 (North), 2 (North-East), 3 (East), 4 (South-East), 5 (South), 6 (South-West), 7 (West), and 8 (North-West). The slope is represented by 1 (-1°-3°), 2 (3°-8°), 3 (8°-13°), 4 (13°-18°), 5 (18°-23°), 6 (23°-28°), 7 (28°-33°), 8 (33°-38°), 9 (38°-43°), 10 (43°-48°), and 11 (48°-53°). Red, blue, and teal colors are used to indicate the values for average, maximum, and without variable in aspect and slope.

topographical variables (Table 1). Notably, the topographic factors contributed more (68.0 %) than the climatic factors (32.0 %) to the model (Fig. 2a, c, and d). Overall, elevation was the most influential predictor (51.4 % contribution) of the distribution of *N. scrophulariiflora*, and the estimated probability of occurrence was high at elevations of around 4000–4400 m but decreased on either side of this range. The probability of occurrence was particularly high on slopes facing north-east and east, and with slope angles of 18–28° and 33–38°. The contributions to predicting the distribution were 8.7 % and 8.2 % for slope and aspect, respectively (Fig. 2c).

Among the seven climatic variables, ‘maximum temperature of warmest month’ (bio5) turned out to contribute the most (second-most of all predictors) to the model (14 %). Another important temperature-based variable was ‘isothermality’ (bio3), which contributed 6.2 %. The probability of occurrence peaked at bio5 \approx 13 °C (Fig. 2b) but remained high within a range around this temperature. Among the precipitation-dependent variables, ‘precipitation of coldest quarter’ (bio19) contributed approximately 4% to the model and showed a peak value at 10 mm. The habitat suitability was also high at much higher precipitation levels and showed a second flat top at around 140–160 mm (Fig. 2f). The habitat suitability was also influenced by other temperature or precipitation variables, but their contributions to the model were relatively low (bio18: 2.9 %, bio15: 2.7 %, bio14: 1.5 %, bio4: 0.5 %). Jackknife tests of the importance of different variables showed that elevation, bio5, and bio18 (precipitation of warmest quarter) had the highest training contributions (>0.3) when used in isolation (Fig. A.1).

3.2. Current potential habitat suitability

The estimated area, potentially suitable for *Neopicrorhiza scrophulariiflora* under recent climatic conditions (1970–2000) was 11,616.5 km² (ca. 7.9 % of the total area of Nepal), with suitable

habitats confined to 28 districts in the high mountains of Nepal (Fig. 3, Table 2). Dolpa district in western Nepal had the highest suitable area of 1436.0 km², corresponding to \sim 1.0 % of the total area of the country, followed by Taplejung in the east (1256.8 km²; 0.9 %). The highest percentages of suitable area were found in Manang in the central part of Nepal (34.6 %), and Taplejung (34.5 %) and Sankhuwasabha (29.5 %) in the east. The central region (all districts of central Nepal) had the largest suitable area (5351.6 km², 3.6 %), followed by the eastern (3218.3 km², 2.2 %) and the western (3046.6 km², 2.1 %) regions (Table A.4). Due to the variation in the width (145–241 km) of the Himalayan range within Nepal in combination with the variation in precipitation, the potential habitat area was observed to widen from east to west, whereas the suitability decreased and the distribution of suitable areas became more fragmented towards the west (Fig. 3). Highly suitable habitats covered a total of 386.3 km², most of which (87.1 %) was concentrated in eastern Nepal. Areas of medium suitability covered about 2787.6 km², predominantly in eastern and central regions, and areas of low suitability covered 8442.6 km², which were distributed from east to west in the higher mountains (Table 2).

There are eleven protected areas (PAs) in the high mountains. Highly suitable habitat areas of *N. scrophulariiflora* were located within five of these PAs spanning across 14 districts. From east to west these were: Kanchenjunga Conservation Area (KCA), Makalu Barun National Park (MBNP), Sagarmatha National Park (SNP), Gaurishankar Conservation Area (GCA), and Annapurna Conservation Area (ACA) (Fig. 3). Nine PAs spanning across 13 districts included areas of medium suitability. In addition to the five already mentioned PAs, these were Langtang National Park (LNP), Manaslu Conservation Area (MCA), Dhorpatan Hunting Reserves (DHR), and Shey-Phoksundo National Park (SpNP), mainly located in the eastern and central highland districts of Nepal. Furthermore, areas of low suitability occurred in two additional PAs, namely Rara National Park (RNP) and Api-Nampa Conservation Area (ANCA).

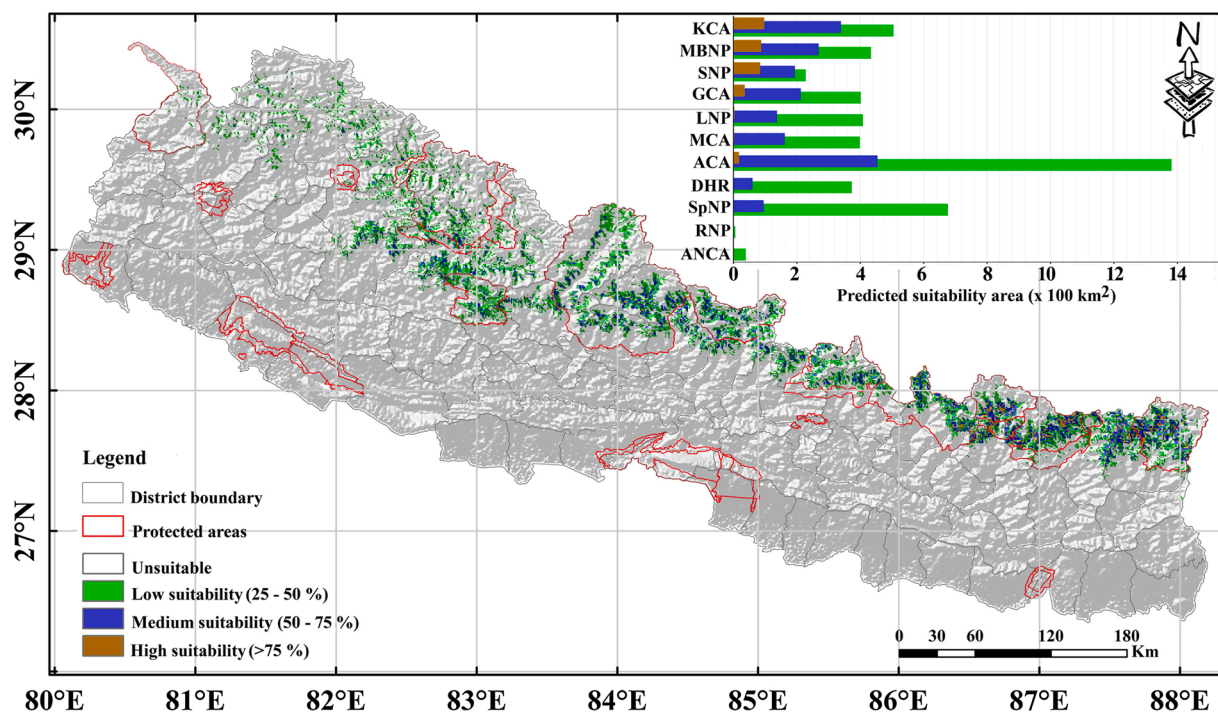


Fig. 3. Habitat suitability for *Neopicrorhiza scrophulariiflora* in the Nepalese Himalayas including protected areas. The suitability levels are specified as low (25–50 %), medium (50–75 %), and high (>75 %). Protected areas with suitable habitat areas are Kanchenjunga Conservation Area (KCA); Makalu Barun National Park (MBNP); Sagarmatha National Park (SNP); Gaurishankar Conservation Area (GCA); Langtang National Park (LNP); Manaslu Conservation Area (MCA); Annapurna Conservation Area (ACA); Dhorpatan Hunting Reserves (DHR); Shey-Phoksundo National Park (SpNP); Rara National Park (RNP); and Api-Nampa Conservation Area (ANCA).

Table 2

Estimated area of habitats suitable for *Neopicrorhiza scrophulariiflora* across districts in the Nepalese Himalayas.

District	Low (km ²)	Medium (km ²)	High (km ²)	Total suitable area (km ²)	Suitable area in % of district area	Suitable area in % of Nepal's area
Western Nepal						
Baglung ^a	115.96	25.60	0.00	141.56	7.94	0.10
Bajhang ^{ab}	140.06	3.01	0.00	143.07	4.18	0.10
Bajura ^a	110.69	3.76	0.00	114.46	5.23	0.08
Darchula ^{ab}	279.36	153.61	13.55	446.53	16.81	0.30
Dolpa ^{ab}	1196.51	239.45	0.00	1435.97	18.20	0.98
Humla ^{ab}	507.52	19.58	0.00	527.10	9.32	0.36
Jajarkot ^a	119.73	36.90	0.00	156.62	7.02	0.11
Jumla ^{ab}	378.76	42.92	0.00	421.68	16.66	0.29
Kalikot ^a	14.31	0.00	0.00	14.31	0.82	0.01
Mugu ^a	342.61	24.85	0.75	368.22	10.42	0.25
Rolpa ^a	0.75	0.00	0.00	0.75	0.04	0.0005
Rukum East ^{ab}	262.04	45.18	0.00	307.22	26.46	0.21
Rukum West	54.22	21.84	0.00	76.05	6.27	0.05
Central Nepal						
Dadhing	100.15	32.38	0.00	132.53	6.88	0.09
Dolakha ^{ab}	279.36	153.61	13.55	446.53	20.38	0.30
Gorkha ^{ab}	578.30	219.12	2.26	799.68	22.15	0.54
Kaski ^b	177.71	67.77	3.76	249.24	12.36	0.17
Lamjung ^{ab}	164.91	58.73	0.00	223.64	13.22	0.15
Manang ^b	511.29	251.50	13.55	776.34	34.57	0.53
Mustang ^b	647.58	137.80	0.75	786.13	22.00	0.53
Myagdi ^{ab}	341.86	71.53	3.01	416.41	18.13	0.28
Ramechhap	69.28	39.16	20.33	128.76	8.33	0.09
Rasuwa ^{ab}	304.21	93.37	0.75	398.34	25.80	0.27
Sindhupalchok ^{ab}	237.19	82.83	2.26	322.28	12.68	0.22
Eastern Nepal						
Panchthar	5.27	0.75	0.00	6.02	0.49	0.004
Sankhuwasabha ^b	615.95	331.32	79.82	1027.09	29.51	0.70
Solukhumbu ^b	406.62	329.81	125.75	862.18	26.03	0.59
Taplejung ^b	697.28	439.00	120.48	1256.75	34.47	0.85

District area is calculated based on statistics provided by the Government of Nepal (GoN, 2012). Symbols: ^a denotes the occurrence of trade and ^b availability (based on herbarium records) of the species.

(ANCA) spanning across three districts of western Nepal (Fig. 3, Table A.5). Protected areas covered about 61.2 % (7108.3 km²) of the total estimated area of suitable habitats. The remaining part of the projected distribution was located outside protected areas (Fig. 3, Table A.6). Comparing the estimated habitat suitability within different PAs, KCA had the largest area (97.1 km²) categorized as highly suitable and ACA had the largest overall area (1854.6 km²) of suitable habitats. RNP only included areas of low suitability and the predicted habitat area (6 km²) was lower than for any other PAs, presumably due to its small size (106 km²) and location at low elevation (Table A.6).

3.3. Trade system and composition

Neopicrorhiza scrophulariiflora was traded from 20 districts (13 with and 7 without recorded occurrences of the species based on the herbarium survey), mostly in western Nepal (Fig. 4a, Table 2). The total traded volume for the 12 fiscal years, 2004/05 – 2015/16, was 372 tons. There was no significant correlation between the traded volume (12 years) for districts and the predicted distribution of areas of high and medium habitat suitability. Also, a positive association ($r = 0.642$, $p = 0.002$) between the traded volume and areas of low suitability indicates that the resource exploitation patterns did not align well with the predicted occurrence of *N. scrophulariiflora* populations. At the same time, annual trade frequency across the 12 fiscal years (2004/05 to 2015/16) showed positive association with areas of low ($r = 0.604$, $p = 0.005$) and medium ($r = 0.473$, $p = 0.035$) suitability but there was no significant association with the area of high suitability. These patterns seem to indicate that exploitation was mainly high in areas with limited resources.

The average annual trade for the 12-year period was 31 tons, varying between a minimum of 10.3 tons (2012/13) and a maximum of 59.9 tons (2015/16), and showed an overall increasing trend (Fig. 4b). The frequency of reported trade was highest for Dolpa (11 out of 12

fiscal years), followed by Lamjung (8 out of 12 fiscal years). The average annual traded volume was highest in Humla (20.6 tons), followed by Dolpa (6.9 tons). The total traded volume over the 12 fiscal years was highest in Dolpa (75.0 tons), followed by Rukum East and Rukum West (62.0 tons, formerly Rukum District), and the least volume (<1 ton) was traded from Surkhet and Dailekh Districts (Fig. 4a).

Western Nepal contributed 28.7 tons (92.7 %) of the annual traded volume, central Nepal contributed 2.3 tons (7.3 %) and, surprisingly, no trade was recorded from eastern Nepal. Nine out of 10 districts of the Karnali Province (Humla, Mugu, Dolpa, Jumla, Kalikot, Dailekh, Jajarkot, Rukum-west and Surkhet) contributed substantially with an average annual trade of 22.9 tons (73.8 % of total), showing the dominance in the trade of *N. scrophulariiflora* in Nepal. However, among the top six districts in terms of suitable habitat area (Dolpa, Taplejung, Sankhuwasabha, Solukhumbu, Gorkha, and Mustang) only Dolpa is located in the Karnali Province (Table 2).

3.4. Market-trend and revenue

We compared the prices of *Neopicrorhiza scrophulariiflora* recorded from 2012 to 2016 at six trade stations: Nepalgunj and Kathmandu in Nepal, and Tanakpur, Delhi, Lucknow, and Kolkata in India. The unit prices were considerably lower in Nepal (mean \pm SE: 10.8 \pm 3.81 USD/kg) than in Indian markets (16.3 \pm 2.88 USD/kg), likely due to factors such as transportation, storage, intergovernmental custom charges, rent-seeking, and central wholesalers' margins (Fig. 5). The average price in Nepal increased from USD 6.3/kg in 2012 to USD 17.1/kg in 2014 but decreased again to USD 9.4/kg in 2016. Over the same period, prices in India were higher but otherwise followed a similar trend, increasing from USD 9.8 to USD 28.4 and then decreasing to USD 12.8/kg. The average price difference between trade stations in Nepal and India was thus little more than 50 % in 2012–2016. The average annual values of domestic trade and export (to India) of *N. scrophulariiflora* were USD

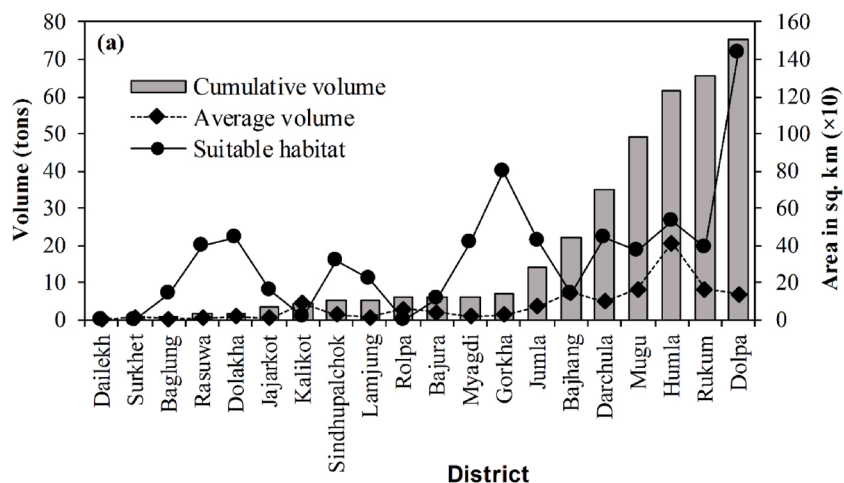


Fig. 4. Trade of *Neopicrorhiza scrophulariiflora* in the Nepalese Himalayas: (a) Cumulative volume traded in the fiscal years 2004/05 to 2015/16, district-wise area of suitable habitats, and average annual traded volume; (b) Volume traded in 2004/05-2015/16, and percentage contribution of the study species by volume and value to total trade of medicinal plants in Nepal. The area of suitable habitat was predicted by the model (cf. Fig. 3). Trade data were obtained from Department of Forest, Government of Nepal.

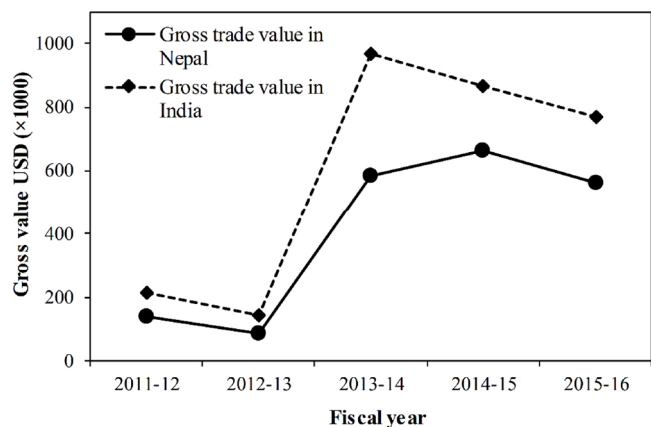
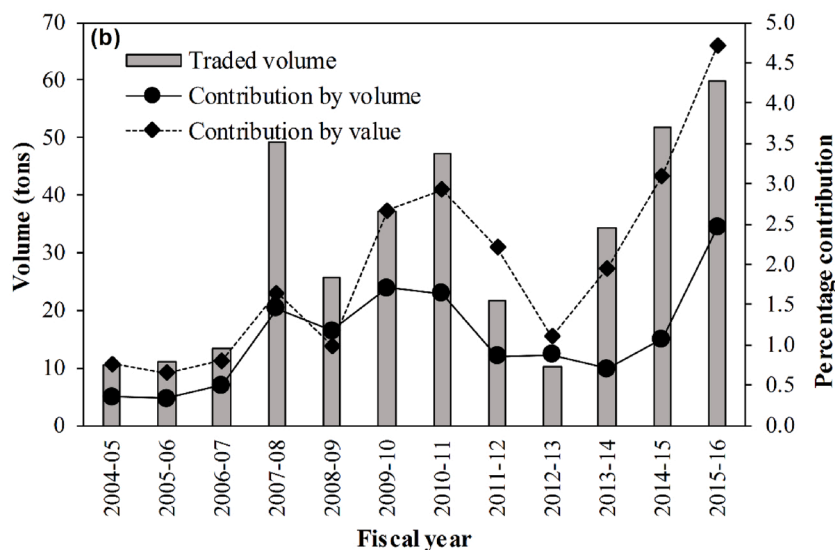


Fig. 5. Total gross value of *Neopicrorhiza scrophulariiflora* traded from Nepal and in India (international markets) during the last five fiscal years, 2011/12–2015/16 (Source: Department of Forest, Government of Nepal).

406,638 and USD 591,570, respectively (Fig. A. 2).

Between 2004/05 and 2015/16, *N. scrophulariiflora* contributed up to 5% (mean ± SE = 1.96 ± 0.34 %) of the total annual government revenue on MAPs and up to 3% (mean ± SE = 1.09 ± 0.17 %) of the total traded volume (Fig. 4b). The average annual revenue collected from the *N. scrophulariiflora* trade was USD 4997 with a minimum of

USD 1322 in 2012/13, and a maximum of USD 9479 in 2010/11.

4. Discussion

4.1. Environmental factors determining habitat suitability

Elevation was the most important determinant of the distribution of *Neopicrorhiza scrophulariiflora*. Our results showed that the species had a very narrow optimal range (4000–4400 m) of occurrence. Above this range, the decrease in predicted habitat suitability might be related to a negative effect of increasing solar irradiance towards higher elevations, possibly leading to UV damage to the photosynthetic apparatus and assimilatory reduction. Below the optimal range, plants might be affected by temperature-dependent physiological constraints, possibly related to accelerated stomatal conductance, high evapotranspiration, and dehydration (Körner, 2003; Lütz and Seidlitz, 2012). For example, plants may experience increased photo-respiration relative to carbon assimilation, as a higher temperature rises the solubility of oxygen and reduces the concentration of photosynthetic (carbon acceptor) enzymes (Körner, 2003; Larcher, 2012). In general for high alpine areas, low temperature, strong wind and desiccation, and a short growing season are the major constraints for plant growth (Körner, 2003; Poudeyal and Ghimire, 2011; Chapagain et al., 2019; Applequist et al., 2020). Based on our model, increasing temperature in the growing season (July/August) appeared to enhance habitat suitability but, nonetheless, suitability decreased again in areas warmer than 13 °C. The specific

combination of rainfall and temperature at the peak of the growing season might have a significant role in activating photosynthetic enzymes, thereby increasing the net photosynthesis per unit area and, hence, facilitating the development of leaves and stems (Larcher, 2012).

In addition to the elevation, topographic variables like aspect (in general north/north-east slopes of the mountains) and slope had significant effects on predicted habitat suitability (Fig. 2a, c, and d). In agreement with this, previous studies (Ghimire et al., 2005; Shrestha and Jha, 2009) reported that *N. scrophulariiflora* populations were generally found on the northern slopes and that their existence depended on the annual melting of snow. Other studies in the Himalayas indicate that south- and west-facing slopes receive particularly high solar irradiation for longer diurnal periods and are thus considered to be comparatively hot and dry (Chhetri et al., 2017; Panda et al., 2017). The slope of the terrain (13–28° and 33–38°, Fig. 2b) showed a positive association with the occurrence of *N. scrophulariiflora*, indicating that the most suitable habitats exist neither in flat nor in too steep areas. Increasing steepness reduces snow cover, which reduces water percolation in the soil. Conversely, on flat land, more snow accumulates and remains for a longer time in spring, thus reducing the number of growing days (Larcher, 2012). On northern slopes, slower melting rates could provide moisture for a longer period of the growing season.

4.2. Regions for predicted distribution and potential cultivation

The MaxEnt ecological niche model showed that highly suitable habitats for *Neopicrorhiza scrophulariiflora* cover a limited area (~0.3 % of the total area of Nepal) of subalpine–alpine zones in central and eastern Nepal. We found higher predicted habitat suitability in the eastern region of Nepal than in regions further towards the west. In agreement with this, the area of suitable habitats decreases towards the western region where suitable patches are more scattered and the overall north-south width of the distribution increases compared to the central and eastern regions. The western part of the country receives low annual (overall) precipitation and frequently experiences droughts, even though overall winter rainfall is slightly greater than in the eastern and central regions (Miehe et al., 2001; Böhner et al., 2015; Chhetri et al., 2017).

N. scrophulariiflora is considered a moisture demanding species and needs adequate precipitation throughout the year (Ghimire et al., 2005; Poudeyal et al., 2019). The precipitation in the coldest (winter, bio19) and in the warmest season (bio18, June/July; growing season) appeared the strongest precipitation-dependent predictors of habitat suitability but were slightly weaker than the temperature-dependent predictors (Fig. 2). This is similar to Poudel et al.'s (2012) findings for *Taxus contorta* Griff., which showed that the distribution of this species is shaped by winter rainfall. In Nepal, winter rainfall enters from the western region, and decreases when it moves towards the eastern region. By contrast, the central and eastern regions (specifically at elevations >2000 m) receive heavier and longer summer monsoon rainfall and experience a smaller number of annual temperature extremes compared to the western region (Poudel et al., 2012; Böhner et al., 2015). The distribution of alpine plant species in the Himalayas of Nepal is widely influenced by the mean annual temperature and precipitation in the dry winter and growing (summer) seasons (Rana et al., 2020; Kunwar et al., 2020).

The higher predicted habitat suitability in the eastern region suggests that this region might be suitable for cultivation and gene pool conservation of *N. scrophulariiflora*. In the western region, Dolpa and Humla have widespread habitat potential, indicating that these districts have relatively high winter precipitation compared to that of other western districts of Nepal (Böhner et al., 2015). Nevertheless, highly suitable areas are not predicted to exist in these districts, possibly due to temperatures slightly higher than the optimum level. Future assessments incorporating a higher number of abiotic (such as soil nutrients) and biotic factors (disturbances) might help to improve prediction of the

habitat suitability in these areas. This may help to identify areas that can be employed in cultivation trials with *N. scrophulariiflora*, an option that appears promising in almost all eastern districts (Table 2) and in Gorkha and Mustang in the central region.

4.3. Discordant trade structure

Our analysis showed that the volume of *Neopicrorhiza scrophulariiflora* rhizomes exported in the fiscal year 2015/16 was six times greater than in 2004/05. The revenue generation also increased almost six times between these two years. With regard to the national consumption of *N. scrophulariiflora*, information about volumes received by domestic industries in Nepal is sparse. Tiwari et al. (2004) reported that 750 kg (price 1.6 USD/kg) of dried rhizomes of *N. scrophulariiflora* was used in the fiscal year 2003/04 by Kathmandu-based companies. A recent study by Kafle et al. (2018) showed that increasing private investments in herbal industries in Kathmandu have increased the domestic consumption by a factor of three (~2000 kg/year, price rate 11.2–15.5 USD/kg). According to the same study, the total amount consumed by Nepalese industries was 6076 kg (Kafle et al., 2018). However, the domestic consumption remained 10 times lower than the export volume. Ghimire et al. (2016) found that the export value of traded MAPs, including *N. scrophulariiflora*, increased from USD 27 million in 2005 to USD 60 million in 2014 due to increased unit cost prices. Also, their evidence showed that resource exploitation is mostly confined to a limited number of high-value MAPs, such as *N. scrophulariiflora*. The increasing trade and prices appeared to be linked mainly to the economic growth and purchasing power of the neighboring countries, India and China (Pyakurel et al., 2018).

The average annual traded volume of *N. scrophulariiflora* from 20 districts was 31 tons according to the DoF data used in this study, but this appears to be far less than the actual quantity traded. Pyakurel et al. (2018) recorded the trade of almost 19 tons from Darchula district alone. Darchula only includes 446.5 km² of suitable habitats (about 4% of the total suitable area in Nepal), whereas the other districts of Nepal have 11547.2 km² (96 %). This indicates that *N. scrophulariiflora* is likely available in other districts in much larger quantities. It therefore seems safe to assume that *N. scrophulariiflora*, with its high international demand and estimated wider availability in Nepal, is traded in far higher quantities than what is registered in government data. Pyakurel et al. (2018; 2019) documented the trade of government banned medicinal plants, showing that products in high demand are generally traded. Olsen (2005) recorded the trade of 529 tons from Nepal in 1997/98, further strengthening the assumption.

There are several possible reasons for the limited documentation of the traded amount; (1) *N. scrophulariiflora* is distributed in high-mountain districts, mostly in protected areas where DNPWC, rather than DoF which is the source of our data, has the management authority; (2) divisional forest office (former district forest office) personnel may be reluctant to forward the trade data to DoF as they are not offered economic incentives to do so (Pyakurel, 2020); (3) traders often underreport the traded volume to reduce royalty payments or to conceal volume exceeding the trade quantity legally permitted by the authorities. Such types of underreporting have been observed elsewhere in Nepal (Olsen, 2005; Devkota et al., 2017; Pyakurel et al., 2018).

Officially traded quantity and availability of medicinal plants are not often correlated as multiple factors determine the reported trade. There are no official records of the trade from eastern Nepal even though the three Himalayan districts (Taplejung, Sankhuwasabha, and Solukhumbu) in this region have substantial areas of high suitability (Table 2). The lack of official trade is most likely due to; (1) collection of forest products from national parks is banned by law and both MBNP and SNP are located in the highlands of Sankhuwasabha and Solukhumbu districts where the suitable areas are found; (2) KCA in the highlands of Taplejung has its own management unit and data are not transferred to DoF. However, this does not rule out the possibility of

trade from eastern Nepal. Ghimire (2009) mentioned the centuries-old trade history of medicinal plants trade from Walangchung Gola of eastern Nepal to Tibet and reported an annual trade of six tons of *N. scrophulariiflora* rhizomes from Taplejung district between 1996 and 2004. Likewise, Pyakurel (2008) reported the undocumented trade of 10–12 tons of fresh plant material from Taplejung to Tibet.

Compared to eastern Nepal, the trade from western Nepal is more well-organized because of a centuries-old trade system. Nepalgunj has been an MAP trading hub for centuries and most of the exporters operate from this town. As a result, a well-established, trust-based trade network, rather than a formal and legitimate system, exists and connects town, villages, and alpine meadows and enables the export of huge quantities of MAPs (Hamilton, 2004; Pyakurel et al., 2018). Similarly, a dependency on collection and trade of medicinal plants, including *N. scrophulariiflora*, for cash income in the western mountain regions could be another important reason for the higher trade from western Nepal compared to eastern Nepal (Hamilton, 2004; Kunwar et al., 2013; Devkota et al., 2017).

Official records from DoF showed that *N. scrophulariiflora* is traded from 20 districts. These 20 districts include districts like Surkhet where the species is not available; or Panchthar, Rolpa and Kalikot with very limited suitable areas (Table 2). The reason for this could be as follows: local traders transport MAPs based on a transport permit, which is valid for 21 days and is issued by the district of origin. In some cases, e.g. caused by natural calamities, traders have to store MAPs for prolonged periods *en route*, and the transport permit expires. In such cases, traders receive a new permit from another district, resulting in the documentation of trade from districts where MAPs are not available.

5. Conclusion and recommendation

We found that about 8% of the area of Nepal, located in the high mountains, is potentially suitable habitat for *Neopicrorhiza scrophulariiflora*, but areas of high suitability (<1%) are limited to a narrow zone of elevation (4000–4400 m). Due to the rainfall patterns, a significant proportion of the most suitable habitats are located in the eastern mountains, but areas in other parts of the country could also be considered for the development of organized cultivation. The trade in *N. scrophulariiflora* rhizomes represents a substantial economic contribution (up to 5% of the value) to the national herbal market and thereby also provides cash income to rural communities, particularly in western Nepal. Nevertheless, the concentration of the most suitable areas in the eastern region in combination with the increasing trade from the western region, which has limited suitable areas, indicates that the resource exploitation is likely to be unsustainable. This supports the hypothesis that overexploitation and high trade in areas that are climatically less suitable for the species might cause local depletion of *N. scrophulariiflora*, which will negatively affect rural livelihoods in western Nepal. However, Dolpa and Mugu districts in the western region receive relatively high winter rainfall and could be considered for experimentation with cultivation practices to supplement the natural resource base.

The potential distribution map that we developed in this study could be used as a baseline when developing management policies for conservation of threatened *N. scrophulariiflora* habitats. Our estimated areas of suitable habitat are based on ecological niche modeling and are largely determined by climatic factors. We therefore suggest conducting more rigorous empirical assessments of possible *N. scrophulariiflora* locations. In addition to climatic factors, there may be other important factors that determine the actual distribution of the species, demanding empirical resource assessment data to quantify the harvestable amount. Comparing empirical resource assessment data with model predictions appears to offer a promising pathway for future research. The present analysis indicates that the eastern and central mountain regions hold considerable potential for *N. scrophulariiflora* harvesting. However, caution is essential, and we therefore suggest conducting rigorous empirical studies on the growth and harvest response of plant

populations as a basis for identifying the optimal annual harvest and developing sustainable management strategies.

Authors' contributions

MRP: Conceptualization; Methodology design; Data collection, curation, and analysis; Writing original manuscript. DP, SKR, and YRP: Data collection and analysis; Review and editing. SKG and HM: Supervision; Conceptualization; Methodology design; Data collection; Funding acquisition; Validation; Project administration; Review and editing.

Data availability statement

All data generated or analyzed during this study are included in this manuscript and the electronic supplemental material. Raw data for the trade of study species are available from the Department of Forest, Government of Nepal. The study species is highly exploited for trade and, therefore, to protect the populations, geo-coordinates are provided only in 'kml' supplemental file (Appendix B), and unprocessed data are not made publicly available. However, for specific research purposes, such data can be made available upon request.

Declaration of Competing Interest

None.

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Appendix A. [(Appendices A and B)] Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jarmap.2021.100292>.

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ORIGINAL RESEARCH

Harvest effects on density and biomass of *Neopicrorhiza scrophulariiflora* vary along environmental gradients in the Nepalese Himalayas

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Abstract

A surprisingly large number of species potentially threatened by human harvest lack quantitative ecological studies incorporating harvest effects, especially clonal species in the alpine Himalayas. We studied density and biomass variation of a threatened medicinal herb, *Neopicrorhiza scrophulariiflora*, to examine the effect of harvest on plant performance. The study covered two regions with contrasting harvest situations—one with open-access and another protected from commercial harvesting. Four populations from each region were compared along an elevation gradient (3,800–4,800 m). Also, we conducted in situ interviews with 165 and 38 medicinal and aromatic plant users in open-access and protected regions, respectively, to assess the collection and use patterns of the target species. The quantity harvested per household for traditional healthcare use was similar in both regions. We found no evidence of trade-driven collection in the protected region but in the open-access region a trade-based annual collection of 35–465 kg dried rhizomes per household had a strong negative effect on both density and biomass. In the protected region, the effect of harvest intensity on plant density was positive for vegetative and negative for reproductive individuals, whereas in the open-access region, the effect was negative for both vegetative and reproductive individuals. The results indicated that a low harvest intensity had no adverse impact on *N. scrophulariiflora* populations; however, quantification of the optimum level of harvest remains to be explored. Shrub vegetation appeared to buffer the harvest impact on plant density, possibly through the retention of additional moisture. To maintain population viability, we suggest regulating harvest, for example, by introducing rotational harvest systems, ensuring that a sufficient number of reproductive individuals are left as a source of propagules in each harvested population and that populations are given time to recover between harvests.

KEYWORDS

clonal herb, conservation, elevation gradient, medicinal plant, shrub facilitation, trade

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1 | INTRODUCTION

Plant life in high mountains, such as the Himalayas, is constrained by biotic and abiotic stress. Contemporary ecological studies from alpine regions primarily emphasize extreme abiotic conditions where plant growth is constrained by short growing season, low temperature, and limited resources (Dvorský et al., 2015; Klimešová, Jiří, Prach, & Košnar, 2012). Interactions with biotic factors, including herbivory, habitat competition, and facilitation, and anthropogenic activities are issues that are not well addressed in plant population studies from alpine areas in the Himalayas (but see Chu et al., 2008, 2009; Ghimire, Gimenez, Pradel, McKey, & Aumeeruddy-Thomas, 2008) and elsewhere (Callaghan, Carlsson, Jónsdóttir, Svensson, & Jonasson, 1992; Körner, 2003). Alpine plant species in the Himalayas are mostly perennial and many of them exhibit clonal growth (Klimeš, 2003; Klimešová, Doležal, Dvorský, Bello, & Klimeš, 2011). Clonal units in such species not only share resources and photosynthates but also absorb negative effects of physical stress, competition, and herbivory, enabling alpine plants to respond to environmental changes (Klimešová et al., 2011; Lei, 2010). At habitat level, the interspecies competition is overlaid by the facilitative role of surrounding vegetation (Ballantyne & Pickering, 2015; Wang, Liang, & Wang, 2014). Delivery of shade and shelter, moisture retention, nutrient accumulation, temperature regulation, and defense against herbivory and physical damage are important services that can be offered by the surrounding vegetation (Ballantyne & Pickering, 2015; Callaway et al., 2002). Thus, the balance between facilitative and competitive environmental interactions has significant ecological implications to the performance of alpine plant species (Chu et al., 2009; Klimešová et al., 2012).

In addition to environmental interactions, alpine plants are in many cases also subjected to high levels of anthropogenic impacts. In the Himalayas, for example, international trade in medicinal and aromatic plants (MAPs) (Olsen, 2005; Pyakurel, Sharma, & Smith-Hall, 2018) has created extreme pressure on populations of some alpine plants and their habitats. In addition, other factors, such as livestock grazing and fire, may reduce densities of alpine plant populations (Niu, He, Zhang, & Lechowicz, 2016). Harvesting of whole plants or plant parts may affect reproduction, survival, and growth, thereby affecting plant population dynamics (Gaoue, Horvitz, Ticktin, Steiner, & Tuljapurkar, 2013; Ghimire et al., 2008; Ghimire, McKey, & Aumeeruddy-Thomas, 2005; Huai, Wen, Xu, & Bai, 2013; Ticktin, 2004). The extent of harvest impact on plant populations, however, varies depending on habitat conditions, plant growth strategies, and species-specific processes, such as regeneration and colonization (Gaoue et al., 2013; Ticktin, 2004, 2015). Clonal plant species to some extent buffer the anthropogenic effects like harvesting by mobilizing stored reserves to maintain normal phases of growth (Mandle & Ticktin, 2012). Furthermore, the clonal tendency toward developing a bud bank after a disturbance also plays a crucial role in vegetative reproduction (Evette, Bédécarrats, & Bornette, 2009). The clonal architecture, especially in plants exhibiting a “guerilla”

strategy of clonal growth, producing long and spreading vegetative offshoots, allows the plant to escape stressful microsites and colonize more suitable ones (Ghimire et al., 2005; Humphrey & Pyke, 1998). In clonal herbs exhibiting a “guerilla” strategy, the density of plants is positively affected by light harvesting if it occurs at sufficiently long intervals and does not exceed the regeneration potential (Ghimire et al., 2005). Thus, with responsible management practices, vegetative propagation in clonal plant species could compensate for reduced biomass caused by anthropogenic disturbances.

The interactions between people and plants in the Himalayas are not yet fully understood (but see Ghimire et al., 2005; Ghimire et al., 2008; Rokaya, Münzbergová, & Dostálek, 2017). Specifically, determining the relative effects of habitat factors, such as substrate, vegetation cover, and disturbance on plant density and biomass is crucial for development of well-informed and effective conservation policies. This study aims to disentangle the factors that control the density and biomass of a clonal medicinal herb, *Neopicrorhiza scrophulariiflora* (Pennell) D.Y. Hong, across a continuum of environmental conditions and harvest intensities in the alpine Himalayas, Nepal. This species is highly threatened due to overharvesting of its rhizome for regional and international trade (Ghimire et al., 2005). Studies emphasizing the variation in density and biomass of such species across a continuum of environmental conditions along an elevation gradient could enhance our understanding of the ability of the species to respond to environmental stress. We will address three questions: (a) In what ways do plant utilization patterns vary between study regions in terms of the purpose and intensity of harvest, and whether harvest takes place in the beginning or at the end of the growing season? (b) How do elevation, surface and vegetation cover, and anthropogenic disturbance influence the density and biomass of *N. scrophulariiflora*? and (c) Does plant response to harvest impact vary between study regions? To answer these questions, we first assessed plant utilization systems and analyzed density, population structure, and biomass variation in different populations along elevation gradients in two study areas characterized by different protection regimes. The species' interaction with environmental factors, including habitat conditions, surrounding vegetation, and human harvest regimes, was analyzed to evaluate these associations.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in two protected areas (hereafter referred to as regions): Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP) in the north-western and north-central parts of Nepalese Himalayas (Figure 1). Both protected areas in those regions are managed by the Department of National Parks and Wildlife Conservation of the Government of Nepal. The Community-led Management Council for ANCA (located in Darchula District) and the national park office for LNP (in Rasuwa

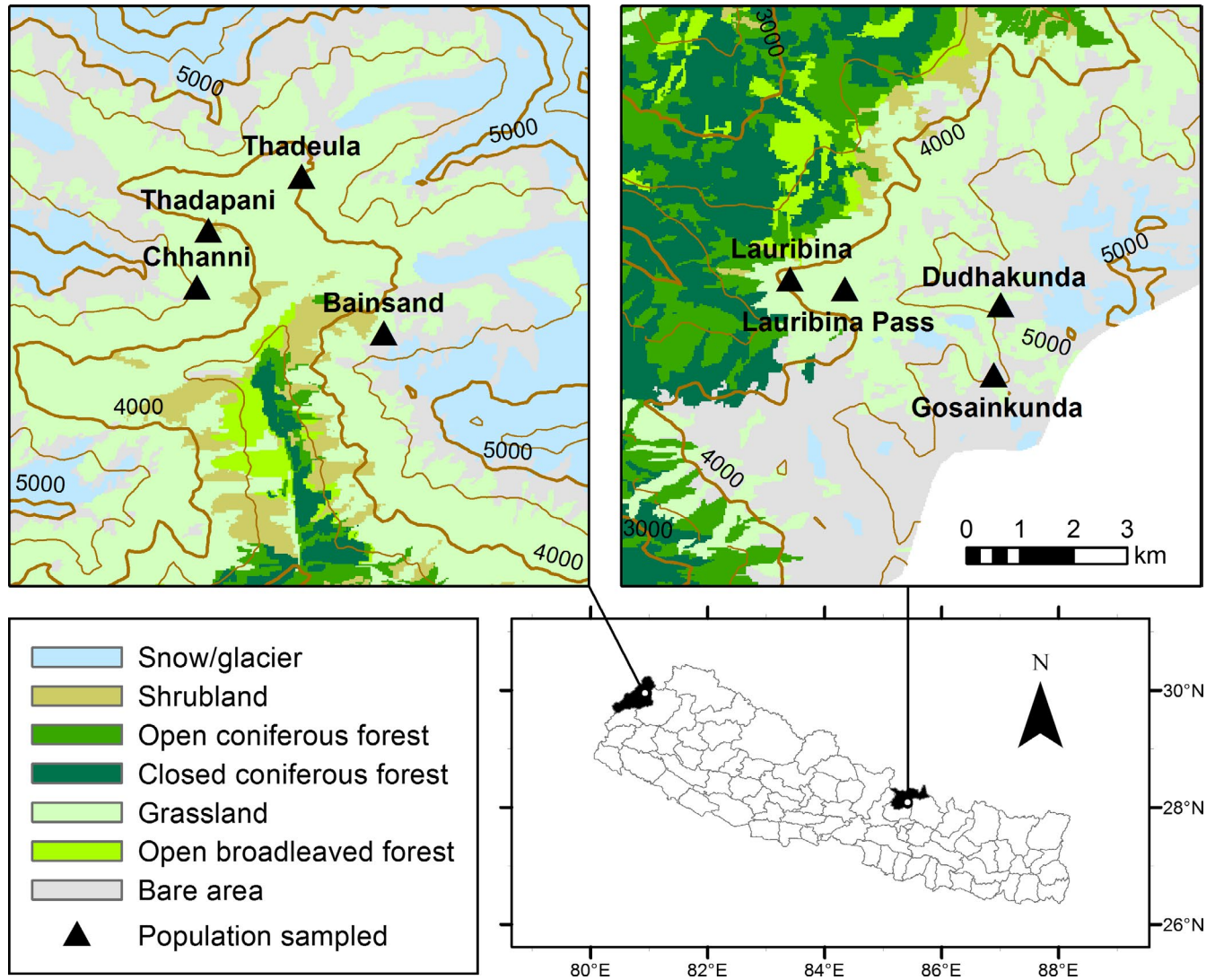


FIGURE 1 Map of the study area: triangles indicate populations included for the ecological study in Api-Nampa Conservation Area (ANCA, left) and Langtang National Park (LNP, right). The thin and thick contour lines are provided to define the elevation scale in every interval of 500 m and 1,000 m from 3,000 to 5,000 m (source of land cover map: Uddin et al., 2015)

District) are the management bodies responsible at the local level. The climate in both regions varies from subtropical to alpine-nival. The two regions are similar in terms of annual precipitation (average of 2,100 mm in ANCA, and 2,300 mm in LNP) and annual average temperature (range: 4–27°C for ANCA and 2–25°C for LNP; both precipitation and temperature were recorded at the nearest meteorological stations located at 1,900 and 1,100 m above sea level, respectively). Both regions are characterized by high mountains and rugged terrain. Almost 70% of residents in ANCA and about 50% in LNP live below the poverty line (Pyakurel et al., 2018; Uprety, Poudel, Asselin, Boon, & Shrestha, 2011). Local livelihoods depend on traditional agriculture, livestock rearing, and seasonal labor (CBS, 2012). The upper slopes are fragile and arable fields are limited to low elevations (<3,000 m), but the productivity is lower in ANCA than in LNP (CBS, 2012; DDC, 2015; Fox, Podger, & Yonzon, 1996). Therefore, people in ANCA supplement their income mostly by harvesting and selling high-valued MAPs

and other forest products (Pouliot, Pyakurel, & Smith-Hall, 2018; Pyakurel et al., 2018). In LNP, people primarily supplement their income through livestock rearing, cheese production, and tourism, and are less involved in harvesting and trade of forest products (Shakya, 2016).

Api-Nampa Conservation Area was recently established in 2010, and in this region commercial harvesting of MAP is legally permitted. The ANCA Management Council issues collection and transportation permits for MAPs (GoN, 2015). Langtang National Park has been protected from commercial harvesting of forest products since its establishment in 1976, and to date, trade permissions have not been issued for any products, except for a high-value medicinal caterpillar fungus, *Ophiocordyceps* (*Ophiocordyceps sinensis* (Berk.) G. H. Sung et al.) (HMG/N, 1978; personal communication with the conservation officer, 26 July 2018). Owing to the differences in MAP harvest and trade regulations, we hereafter refer to ANCA as “open” and LNP as “protected.”

2.2 | Study species

Neopicrorhiza scrophulariiflora (Pennell) D.Y. Hong (hereinafter referred to as *Neopicrorhiza*) of the family Plantaginaceae (previously Scrophulariaceae) (APG IV, 2016) is a perennial, rhizomatous herb that exhibits clonal growth. It is confined to the pan-Himalayas, the Tibetan Plateau, Assam-Burma, and south-central China, and is found at elevations between 3,500 and 4,800 m (Ghimire et al., 2005; Hong, 1998). The plant is relatively slow growing and is mostly confined to cool north-facing slopes (Shrestha & Jha, 2009). Flowering and fruiting occur in June–September. Seedling recruitment is low, and propagation mainly takes place by vegetative means (Ghimire et al., 2005). The modular growth of the individual genet takes place by horizontal extension of ramets (vegetative offshoots), which can survive independently when detached. The clonal growth of *Neopicrorhiza* has been described as a “guerrilla” strategy, leading to spaced clusters of spreading fugitive ramets connected below ground by horizontal rhizomes (Ghimire et al., 2005).

The rhizomes of *Neopicrorhiza* are valued for the treatment of cough, cold, headache, fever, high blood pressure, conjunctivitis, bile reflux, and intestinal pains, among others (Ghimire et al., 2005; Manandhar, 2002). It is one of the most commonly consumed herbs in the Himalayas and is appraised to have a high pharmacological potential (Li, Liu, Abdulla, Aisa, & Suo, 2014). The plant is resistant to grazing, and thus, premature and excessive harvesting is the main issue for its conservation (Ghimire et al., 2005; Shrestha & Jha, 2009). It is closely related to *Picrorhiza kurrooa* Royle, a species native to north-west India listed in CITES Appendix II. Rhizomes of both species are traded globally, but *Neopicrorhiza* is more commonly traded than *P. kurrooa* (Mulliken, 2000). Rhizomes of *Neopicrorhiza* collected from Nepal are mostly traded to India in air-dried form through well-established marketing chains (Olsen, 2005) with a current annual trade volume of ca. 350 tons (personal communication with traders in ANCA and at a major road-head collection center in Nepalgunj, south-west Nepal, November/December 2017).

2.3 | Interviews with local resource users

This work was carried out as a partial basis for the first author's Ph.D. dissertation at the Central Department of Botany (CDB), Tribhuvan University, Nepal. The study proposal was approved by the CDB Research Committee. Written permission for fieldwork was obtained from Department of National Park and Wildlife Conservation, Government of Nepal. The research objectives were explained, and prior informed consent was received from respondents before conducting interviews and consultations with MAP users in ANCA and LNP. The participants were informed they could withdraw their consent at any point in case of disagreement.

Commercial MAP harvesting and livestock grazing are the main human activities in the alpine regions of the Himalayas. Local people usually follow traditional systems of rotational grazing when livestock are brought to subalpine and alpine pastures for summer grazing. We recorded two types of MAP collectors: dedicated

collectors, who collect only one target species at a time and are not involved in pastoralism; and opportunistic collectors, who collect different MAP species and are also involved in pastoralism (Olsen, 1998). On average across the grazing seasons of 2015–2017, we recorded 1,500 livestock (100 cattle and 1,400 sheep/goats) and 30 herders in ANCA, and 1,000 livestock (200 cattle and 800 sheep/goats) and 20 herders in LNP at the study locations (based on interviews with herders, 2015–2017). Commercial harvesting of *Neopicrorhiza* usually takes place during the late part of the growing season (August–October). Apart from this, in ANCA, harvesting of *Neopicrorhiza* usually coincides with the harvesting season of *Ophiocordyceps* in late May to early July. We used in situ open interviews with 165 MAP users in ANCA and 38 in LNP to solicit information about harvesting and utilization. We assessed purpose and level of harvest of *Neopicrorhiza* by asking four questions: (a) Do you harvest *Neopicrorhiza*? (c) If yes, then for what purpose (only for domestic consumption or also for selling)? (c) Which season do you prefer to go for collection? and (d) How much quantity do you collect typically in a year?

2.4 | Plot establishment and sampling

We carried out a sample-based study in *Neopicrorhiza* populations during the peak growing season (June–August of 2015–2017). We identified four large populations of *Neopicrorhiza* in each of the two regions, within an elevation range of 3,500–4,800 m along upper Chameliya valley of ANCA and upper Trishuli watershed of LNP (Figure 1, Table S1). In each population, we laid out three transects at intervals of 100 m (Ghimire et al., 2008, 2005). In each transect, we established two plots (3 × 3 m) at horizontal distances of at least 20 m from each other, depending on field conditions. The clonal behavior of the study species is such that sprouting occurs readily from the parental ramet within a limited distance leading to a patchy form of distribution. Therefore, we established the plots subjectively where the plants occurred in sufficient number for sampling (Whittaker, 1956). We divided each plot into nine subplots (1 × 1 m). Among these, five subplots (four at the corners and one at the center) were systematically sampled and measured. In essence, the study included 48 plots with 240 subplots, and each of the samples in the two regions included half of these.

We studied plant density for ramet stage classes. In each subplot, we classified individual ramets, based on number and size of leaves and occurrence of reproductive organs, into one of the three stage classes (Oostermeijer, Brugman, Boer, & Nijs, 1996; Weppler, Stoll, & Stöcklin, 2006): (a) juvenile ramets bearing 1–4 smaller-sized (range: 0.5–2.0 cm length) leaves; (b) adult vegetative ramets bearing more than four larger-sized (>1.5–9.0 cm length) leaves but with no indication of sexual reproduction; and (c) reproductive ramets bearing any number and size of leaves and a reproductive peduncle. To describe population structure, we calculated the proportion of ramets in each stage class.

For the estimation of harvestable rhizome biomass, we asked local harvesters to collect 2–6 full-grown ramets (adult vegetative and reproductive stages only) from eight to eighteen 1 × 1 m plots

in each population, laid out randomly in the vicinity of plots used in density estimation. This way we collected biomass samples from 50 subplots in ANCA and 46 in LNP. In total, we extracted 161 biomass samples in ANCA and 133 in LNP (30–59 samples including above- and below-ground parts per population). We measured the length and girth of each rhizome when fresh. Girth was calculated as a mean of three measurements at different nodal points along the rhizome. The volume of each rhizome was calculated based on girth and length assuming cylindrical shape. The biomass of each rhizome sample was measured after drying for 72 hr at 60°C in a hot air oven. The volume and biomass were used for calculation of rhizome tissue density (expressed in g/cm^3). The total number of rhizomes per kilogram dry weight was calculated for each population based on the estimated mean biomass.

2.5 | Plot-based environmental variables

Geographical location (latitude and longitude) and topographic features (elevation, slope, and aspect) were measured for each plot. Latitude, slope, and aspect were used to calculate potential annual direct incident radiation (PADIR) (McCune, 2007; McCune & Keon, 2002). Vegetation data were recorded in each subplot (Table S2). The shrub canopy cover was estimated visually as total canopy cover of all shrub species, and shrub height was measured at three points located diagonally across the subplot. Cover of ground vegetation was estimated separately for herbs (all dicotyledons species, orchids, and lilies), graminoids (including all grasses and sedges), and lichens/mosses. Similarly, bare ground cover and rock cover were estimated for each subplot. Soil pH was measured using a portable pH meter (Mudder brand, United States of America, accuracy: ± 0.3) in each subplot as an average of three measurements taken diagonally at equidistant points.

Evidence of MAP harvesting and livestock grazing was confirmed by intensive observation in each subplot. In order to determine the magnitude of impact, subplots were further divided into four cells, each 0.25 m^2 in size. We assigned a binary score to each cell based

on whether there was evidence of disturbance (1) or not (0). At the subplot level, the scores given separately for each category of disturbance were combined for the four cells to obtain an overall score ranging from 0 (null) to 4 (very high). The following types of evidence were used in assessing the harvesting of *Neopicrorhiza*: the presence of left-over rhizome fragments, uprooted plants, and holes or scars left after the excavation of plants (Figure 2). Assessment of grazing was based on the presence of pugmarks and trampling, bite marks, browsed plants, broken, defoliated or disorientated aerial parts and animal droppings.

2.6 | Data analysis

Variation in ramet density, biomass, and environmental covariates among populations and between regions was tested by nonparametric Kruskal–Wallis one-way ANOVA and Mann–Whitney *U* tests. A generalized linear mixed effects model (GLMM) was used to examine the effect of environmental factors on ramet density, and a linear mixed effects model (LMM) was used for analyzing rhizome biomass and tissue density. A negative binomial distribution was assumed for ramet density, and rhizome biomass and tissue density were transformed using the natural logarithm to make their residuals normally distributed. To control for intra-class correlation caused by the nested design, “plot” was included as a random effect in models for ramet density. Similarly, a random effect of “subplot” was included in models for rhizome biomass and tissue density. Effects of region and population were treated as fixed nominal factors. All numeric environmental predictors (Table S2) were treated as covariates. These covariates were standardized (mean 0, standard deviation 1) to avoid erroneous conclusions when analyzing interactions between continuous variables and to facilitate the interpretation of variables and models (Bråthen & Lortie, 2016). We used qq-plots to evaluate the properties of residuals in models based on different distribution assumptions (Zuur, Ieno, Walker, Saveliev, & Smith, 2009). In the case of ramet density, the primary additive model was prepared by starting with



FIGURE 2 *Neopicrorhiza scrophulariiflora* growing in (a) heavily harvested and (b) unharvested habitats; an individual plant at fruiting stage is 5–25 cm tall. The approximate size of objects is indicated by the scale bars

a complete model, including all fixed effects and the random plot effect. Then, the model was reduced successively by backward elimination, gradually removing predictors that were not significant ($p \geq 0.05$). Based on the final model with only significant predictors, extended models including two-way interactions between regions, harvest, and elevation were prepared. In the case of rhizome biomass and tissue density, the number of potential predictors was lower and a selection procedure was not applied. For each model, the Akaike information criterion (AIC) was calculated and compared with the null model. The model with the lowest AIC value was considered as the best model. To assess the validity of the selected model, we performed likelihood ratio tests comparing models with fixed effects to the null model. Pearson correlation analysis was used to examine co-variation between response and predictor variables when more elaboration was required. The correlation analysis, Kruskal–Wallis and Mann–Whitney U tests were carried out in IBM SPSS 24 (IBM, 2016), and GLMM and LMM were prepared using the lme4 package in R version 3.5 (R Core & Team, 2017).

3 | RESULTS

3.1 | Variation in utilization pattern

We recorded both opportunistic (26%) and dedicated (74%) collectors in ANCA. About, 5% of MAP users interviewed ($n = 165$) did not harvest *Neopicrorhiza*, 90% harvested it both for trade and local healthcare, and the rest 5% (all traditional healers) collected it only for local health care. Among the MAP users, 7%, 57%, and 31% collected *Neopicrorhiza* before the growth season (May/June), after the growth season (August/September) and both before and after, respectively. A conservative estimate of the annual collection is that on average people from ANCA collected 0.5–3.5 kg (mean \pm SE = 1.56 \pm 0.06 kg) dry rhizomes per household for local health care and 35–465 kg (mean \pm SE = 170.50 \pm 6.64 kg) per household for trade.

We found no evidence of trade-driven collection in the LNP region. In contrast to ANCA, all the MAP collectors interviewed in LNP were opportunistic, 87% (33 of the 38 interviewees) harvested *Neopicrorhiza*, and 88% of them told that the collection was for local health care. Only few users in LNP collected *Neopicrorhiza* during May/June (6%) and the majority collected in August/September (94%). Among the collectors, about 12% indicated willingness to sell the rhizomes should an opportunity arise, which is however unlikely due to the legal restrictions implied by the protected area system. Like in ANCA, people in LNP collected 1.0–3.0 kg (1.89 \pm 0.09 kg) dried rhizome per household annually for local healthcare consumption.

3.2 | Variation in plant density

Ramet density (m^{-2}) varied within and between the regions (Figure 3). Importantly, for total ramet density (all stages combined), LNP populations were 1.5 times denser than ANCA populations (mean \pm SE = 42.93 \pm 3.35 for ANCA, 61.12 \pm 3.83 for LNP, $p < 0.0001$). For individual stage classes, the densities in the two regions decreased in the same order from adult vegetative (33.60 \pm 2.60 and 48.05 \pm 3.07, $p < 0.0001$), over juvenile (7.28 \pm 0.66 and 10.03 \pm 0.77, $p = 0.002$) to reproductive (2.05 \pm 0.31 and 3.03 \pm 0.37, $p = 0.006$, in ANCA and LNP, respectively). In ANCA, only reproductive density varied significantly ($p = 0.004$) among populations and was highest in Thadeula and lowest in Thadapani (Figure 3). Similarly, in LNP juvenile density did not differ much among the populations, but adult vegetative density was highest in Lauribina and lowest in Dhudhakunda ($p = 0.002$), and reproductive density was highest in Gosainkunda and lowest in Lauribina ($p = 0.019$, Figure 3). The population structure in terms of ramet stage class composition clearly deviated between the study regions ($p = 0.023$, Kolmogorov–Smirnov test) with greater reproductive ramet proportion in LNP (0.053 \pm 0.006) than in ANCA (0.033 \pm 0.004, $p = 0.011$). By contrast, the proportions of ramets in other stage classes did not vary between the regions (Figure 3). Also, ramet composition did not vary substantially across populations within each region ($p = 0.267$ for ANCA and 0.656 for LNP).

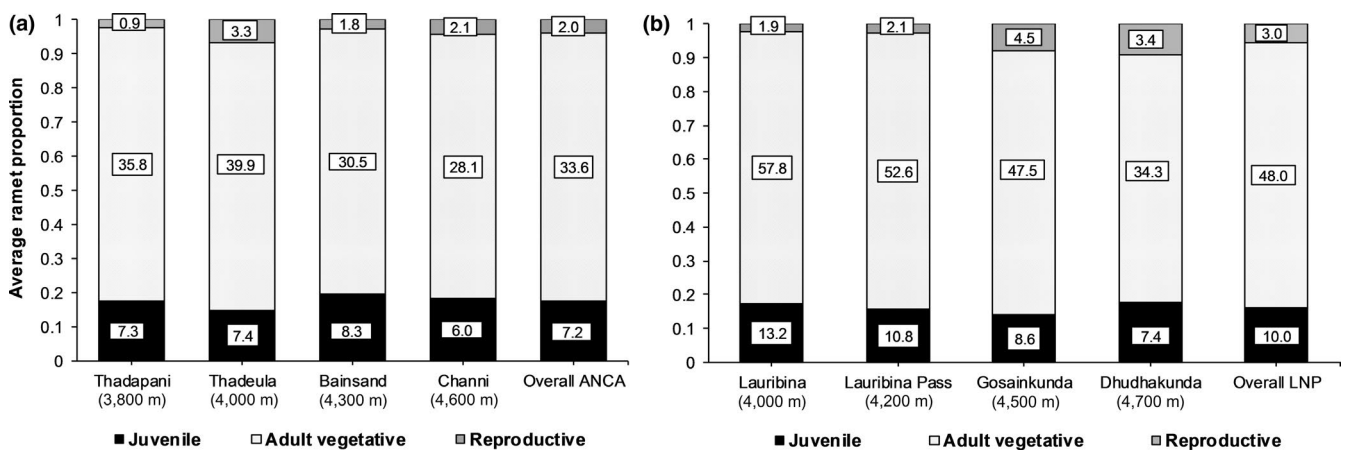


FIGURE 3 Population-wise proportions of different life stages derived from ramet density (mean value (m^{-2}) is included in each part of the columns) of *Neopicrorhiza scrophulariiflora* in two regions: (a) Api-Nampa Conservation Area (ANCA) and (b) Langtang National Park (LNP), Nepal

3.3 | Variation in rhizome biomass

Ramet girth (cm) was higher in ANCA (mean \pm SE = 0.211 ± 0.008) than in LNP (0.176 ± 0.006 , $p < 0.001$). Likewise, mean rhizome volume (cm^3) was also higher in ANCA (0.36 ± 0.05) than in LNP (0.26 ± 0.02 , $p = 0.023$). On the other hand, the rhizome tissue density (g/cm^3) was considerably higher in LNP (5.53 ± 0.37) than in ANCA (2.66 ± 0.22 , $p < 0.001$). In addition, the mean rhizome biomass (dry weight in g) was about 1.5 times higher in LNP (0.74 ± 0.03) than in ANCA (0.47 ± 0.02 , $p < 0.001$). In ANCA, mean rhizome biomass did not deviate between the populations (Figure 4a). Moreover, within LNP, the biomass appeared to be highest for the mid-elevation population, Lauribina Pass at 4,200 m and, otherwise, it decreased with increasing elevation (Figure 4b). In ANCA, an average of 2,136 dried rhizomes (range: 1,964–2,310) were needed to obtain one kilogram; while in LNP, an average of 1,358 rhizomes (range: 1,001–1,780) were required per kg. In both regions, the number of individuals needed to obtain one kilogram of dried rhizome generally increased with elevation.

3.4 | Effect of environmental factors on plant density and biomass

The environmental variables recorded in the sample plots varied within and between regions (Table S2). Major differences between regions were observed for ground cover variables and disturbances. Shrub, rock, and moss/lichen cover were higher in LNP than in ANCA. Cover of herbs and graminoids was higher in ANCA than in LNP. The average scores of harvesting and grazing were remarkably higher in plots studied in ANCA than in LNP (Table S2).

Out of twelve variables (including "population") studied (Table S2), only nine were used in GLMM after removing confounded variables. Moss/lichen cover was confounded with shrub cover, and bare ground cover was confounded with herb and graminoid cover. Shrub height revealed several missing values and so, it was excluded in the model. Generalized linear mixed effects model

identified five variables that exhibited a clear association with plant density (Table 1). Rock cover, in general, did not show any notable effect on plant density when data from both regions were treated together. However, when data were treated separately for each region, rock cover was found to be associated positively with plant density in ANCA (Pearson correlation, $r = 0.323$, 0.281 , 0.320 and 0.311 for juvenile, adult vegetative, reproductive and total ramet density, respectively; $p < 0.010$; Figure 5a). By contrast, in LNP the corresponding relationship was negative ($r = -0.303$ and -0.275 for adult vegetative and total ramet density, respectively; $p < 0.010$; Figure 5b).

In the combined model, shrub cover also appeared to have a positive effect on ramet density (Table 1). When data were treated separately for each region, it emerged that increasing shrub cover was associated with increasing ramet density in LNP (Figure 5d) but not in ANCA. For individual stage classes in LNP, shrub cover showed a weaker positive relation with reproductive ramet density ($r = 0.269$, $p = 0.003$) than with juvenile ($r = 0.398$, $p < 0.001$) and adult vegetative ($r = 0.579$, $p < 0.001$) ramet density. By contrast, in ANCA, the shrub cover showed a negative relationship with ramet density (Figure 5c), but the relationship was only significant for reproductive ramet density ($r = -0.229$, $p = 0.012$).

Among all environmental variables studied, harvest disturbance had a negative effect on ramet density for all stage classes, whereas the effect of grazing was positive for juvenile and adult vegetative ramet densities (Table 1). A significant interaction between harvest impact and region further indicated that the negative effect of harvest on plant density was stronger in ANCA, and the effect of harvest was subtle for the LNP populations (Table 2). The weak positive effect of harvest on ramet density in LNP (positive interaction in Table 2) for all vegetative stages could be linked to the narrow range of harvest intensities.

The harvest impact showed different association patterns with rock and shrub cover in the two regions. In LNP, the harvest score exhibited no clear relation with shrub cover ($p > 0.050$), whereas the

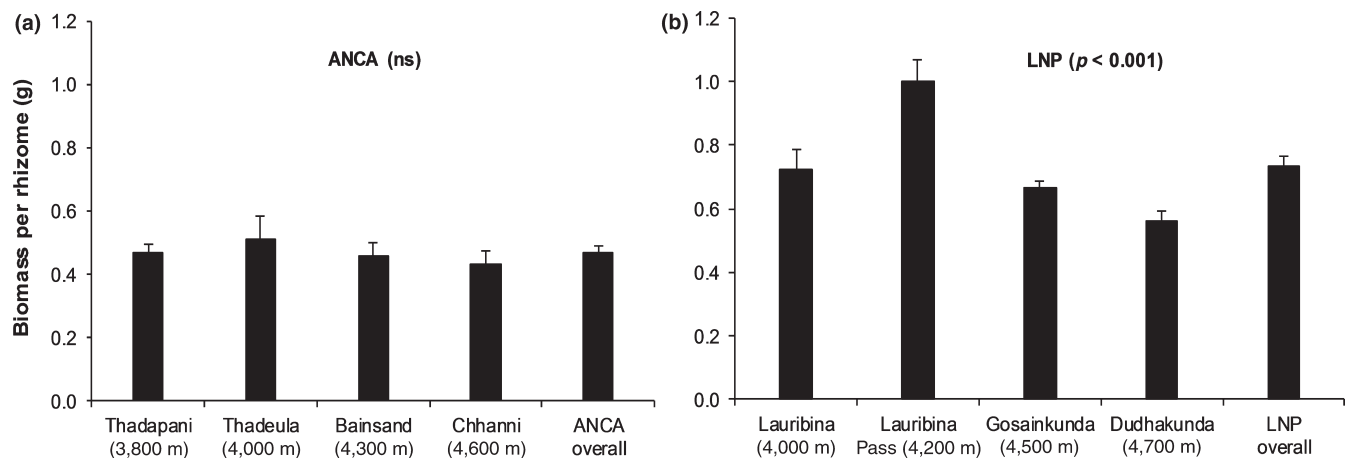


FIGURE 4 Population-wise ramet biomass (Mean \pm SE) of *Neopicrorhiza scrophulariiflora* in (a) Api-Nampa Conservation Area (ANCA) and (b) Langtang National Park (LNP) in Nepal. The variation between populations was tested using the Kruskal–Wallis test (p -values stated in brackets)

TABLE 1 Generalized linear mixed effect models expressing the effects of environmental factors on ramet density (m^{-2}) of *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), Nepal

Explanatory variable	Juvenile		Adult vegetative		Reproductive		Overall	
	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error
ANCA								
Intercept: Thadapani 3,800 m	2.117***	0.180	3.695***	0.153	0.295	0.272	3.923***	0.157
Thadeula 4,000 m	-0.232	0.254	0.22	0.215	0.874*	0.342	0.205	0.22
Bainsand 4,300 m	-0.162	0.254	-0.206	0.213	0.382	0.364	-0.172	0.218
Chhanni 4,600 m	-0.213	0.255	-0.243	0.222	0.203	0.361	-0.258	0.227
LNP								
Lauribina 4,000 m	0.264	0.253	0.16	0.221	-0.24	0.367	0.202	0.226
Lauribina Pass 4,200 m	-0.259	0.263	-0.212	0.230	-0.676	0.369	-0.195	0.236
Gosainkunda 4,500 m	-0.169	0.271	-0.213	0.224	0.117	0.344	-0.189	0.231
Dhuhakunda 4,700 m	-0.346	0.274	-0.467*	0.229	-0.574	0.359	-0.414	0.235
Cover substrate								
Shrub cover	0.230***	0.069	0.281***	0.069	0.294***	0.084	0.260***	0.071
Graminoid cover	-0.237***	0.064			-0.368***	0.098		
Edaphic								
Soil pH	0.157*	0.072						
Disturbance								
Harvest	-0.390***	0.072	-0.556***	0.072	-0.871***	0.088	-0.570***	0.074
Grazing	0.335***	0.07	0.402***	0.071	-0.143	0.08	0.384***	0.072
AIC: Full	1,483.931		2,210.60		865.266		2,331.222	
AIC: Null	1,530.20		2,269.50		973.90		2,385.51	
Observations (n)	240.00		240.00		240.00		240.00	

Note: Models were estimated assuming a negative binomial distribution and "plot" was included as a random factor. Levels of significance are indicated by the symbols: $p < 0.0001$ "****", $p < 0.001$ "***", $p < 0.05$ "**", $p < 0.01$ "*" and $p < 0.05$ "*".

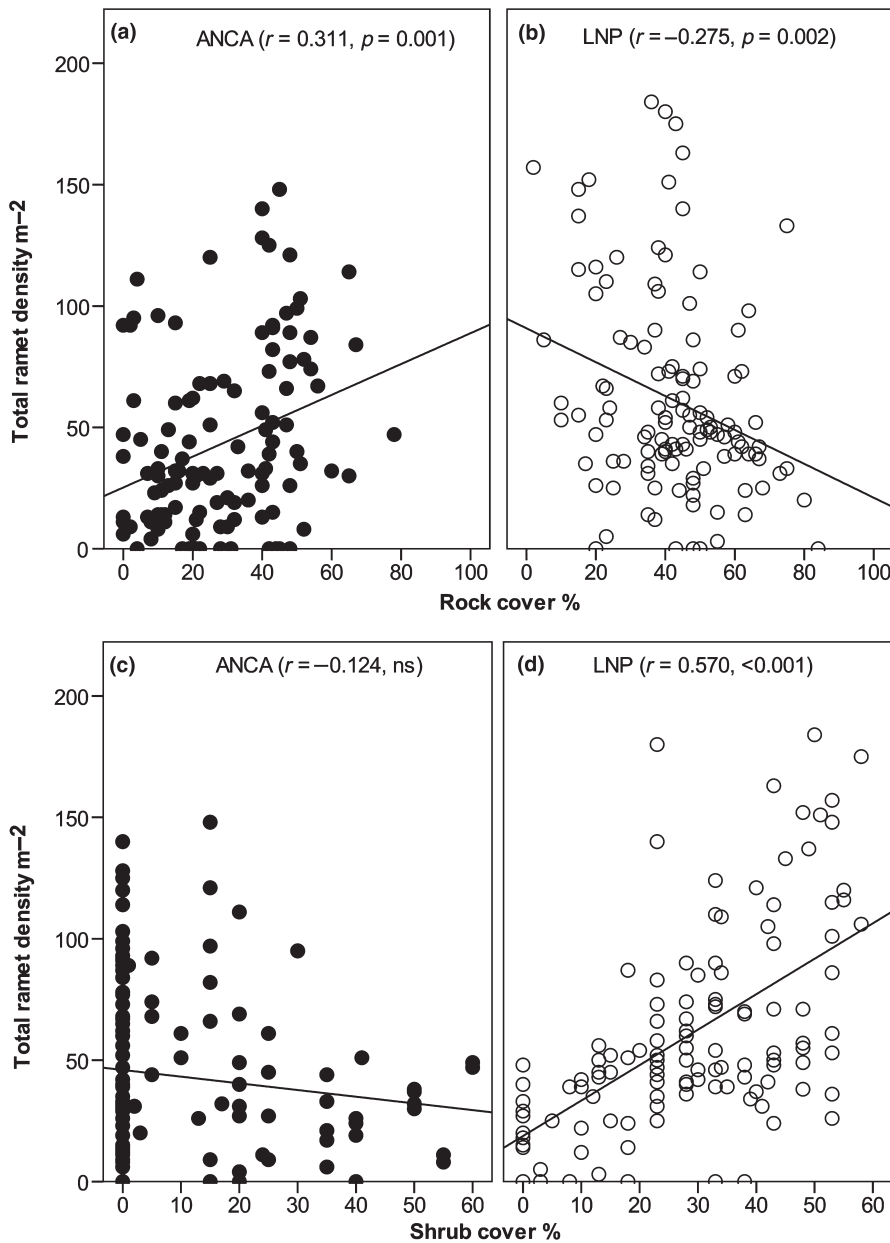


FIGURE 5 Relationship between total ramet density (all stages, m⁻²) and (a, b) rock cover, and (c, d) shrub cover for *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), Nepal

relationship was positive in ANCA ($r = 0.326$, $p < 0.001$). Contrary to this, in ANCA, the harvest impact score was negatively related with rock cover ($r = -0.268$, $p = 0.003$); whereas, in LNP, the relation was not significant. The positive relationship between harvest impact score and shrub cover further indicated that shrubland habitats in ANCA were preferred collecting sites for *Neopicrorhiza* harvesters.

In general, while fitting variables in GLMM (Table 2), we detected a lower value of AIC when elevation (AIC = 2,282.85) was included in models instead of populations (2,287.90, against null model = 2,385.51). This may indicate that part of the variability among the populations can be ascribed to differences in elevation (Table 2). Elevation generally had negative parameter estimates, indicating that ramet density gradually decreases with increasing elevation. In contrast to the other stages, reproductive ramet density in LNP was weakly positively related to elevation (Table 2). Otherwise, the effect of elevation on ramet density was less negative in LNP

than in ANCA, which could be related to the better protection characterizing LNP. Moreover, harvest was heavier at low elevations than further uphill ($r = -0.252$, $p = 0.006$, for ANCA and $r = -0.367$, $p = 0.001$ for LNP), presumably reducing ramet densities at low elevations.

Rhizome girth decreased with increasing elevation but this effect was more prominent in LNP ($r = -0.490$, $p < 0.001$) than in ANCA ($r = -0.162$, $p = 0.040$). Rhizome volume was not correlated with elevation in ANCA, but in LNP, it decreased with increasing elevation ($r = -0.591$, $p < 0.001$). Harvest negatively affected rhizome biomass in both regions and this effect was slightly lower in LNP than in ANCA (Table 2). We did not observe an effect of elevation on biomass (Table 2), but rhizome tissue density increased with elevation in both regions (Table 2). This indicates that tissue density was more sensitive to environmental variation along the elevation gradient than biomass. Additionally, this may suggest that older ramets with

TABLE 2 Generalized linear mixed effect models expressing the effect of environmental factors on ramet density (m^{-2}), ramet biomass (g), and tissue density (g/cm^3) of *Neopicrorhiza scrophulariiflora* in Api-Nampa Conservation Area (ANCA) and Langtang National Park (LNP), Nepal

Explanatory variable	Juvenile		Adult vegetative		Reproductive		Total ramets		Rhizome biomass		Rhizome tissue density	
	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error	Parameter estimate	Standard error
Region												
Intercept: ANCA	1.874***	0.126	3.441***	0.103	0.333*	0.161	3.684***	0.104	-0.889***	0.056	0.814***	0.093
LNP	0.410*	0.173	0.454**	0.140	-0.076	0.226	4.126***	0.099	0.497***	0.084	0.215	0.139
Harvest	-0.667***	0.094	-0.757***	0.100	-1.164***	0.129	-0.762***	0.101	-0.279***	0.053	-0.353***	0.087
Elevation	-0.264	0.135	-0.360**	0.111	-0.435**	0.168	-0.354**	0.112	-0.118	0.060	0.251*	0.101
Interaction												
LNP*Harvest	0.805***	0.123	0.820***	0.131	0.296	0.177	0.796***	0.133	0.079	0.086	-0.044	0.144
LNP*Elevation	0.134	0.178	0.204	0.146	0.524*	0.218	0.211	0.148	-0.078	0.081	0.144	0.136
AIC: Full	1,481.752		2,209.50		865.266		2,326.979		234.20		657.40	
AIC: Null	1530.20		2,269.50		973.90		2,385.51		295.80		717.50	
Observations (n)	240.00		240.00		240.00		240.00		294.00		294.00	

Note: Parameters were estimated assuming a negative binomial distribution for density, and natural log transformation was used to control for the skewed distribution of biomass and tissue density. A random effect of "Plot" was included for ramet density, and for biomass and tissue density of rhizome "Subplot" was included as a random factor in the models. Levels of significance are indicated by the symbols: $p < 0.0001$ "****", $p < 0.001$ "***", and $p < 0.05$ "**".

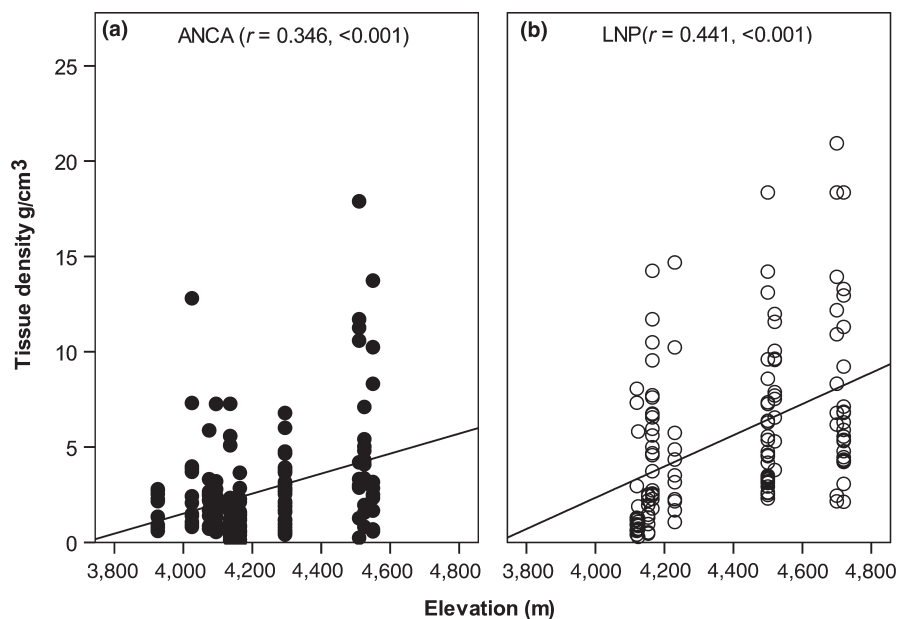


FIGURE 6 Relationship between elevation and rhizome tissue density of *Neopicrorhiza scrophulariiflora* in (a) Api-Nampa Conservation Area (ANCA) and (b) Langtang National Park (LNP), Nepal

denser tissues were more prevalent at higher elevation, especially in populations in LNP (Figure 6a,b).

4 | DISCUSSION

4.1 | Utilization practices and pressure on wild MAP resources

We observed less harvest pressure in LNP (which is protected) than ANCA (which is open-access region) and encountered a lower number of *Neopicrorhiza* users in LNP where all harvest was for local use. By contrast, in ANCA a greater number of users harvested *Neopicrorhiza* for trade than for local use, and the amount harvested per household was higher than reported in earlier studies from the Himalayas (e.g., Ghimire et al., 2005; Uniyal, Uniyal, & Rawat, 2011). For example, Pyakurel et al. (2018) estimated the trade of *Neopicrorhiza* from ANCA to be 18.55 tonnes in fiscal year 2014/2015. The amount harvested for local healthcare was similar in the two regions but, due to the heavy harvest for trade in ANCA, increases in harvest in this region may seriously threaten its *Neopicrorhiza* populations.

In Nepal, *Neopicrorhiza* is one of the high-valued MAPs of the alpine region with a century-long trade history (Olsen, 2005; Olsen & Larsen, 2003). In recent years, the exploitation of herbal and fungal MAPs has become a significant challenge for sustainable resource management in the Himalayas (Cunningham et al., 2018; Negi, Pant, Joshi, & Bohra, 2014). Presently, due to lucrative markets and surging prices, the collection of the highly valued parasitic fungus *Ophiocordyceps* has exceeded the total income from the harvest of all other MAPs (Negi et al., 2014; Pouliot et al., 2018). However, when valuable MAPs become scarce, collectors start collecting other highly valued products (Lange, 2004; Mulliken, 2000).

Since livelihood options in ANCA are constrained by its rugged terrain and remoteness, people supplement their income primarily

by harvesting MAPs and other forest products (Pouliot et al., 2018; Pyakurel et al., 2018). In contrast, LNP is an attractive tourist destination in Nepal, in part because it features an important pilgrimage site (the sacred Gosainkunda lake), which is visited by tens of thousands of devotees every year (Shakya, 2016). People in LNP therefore supplement their income by engaging in tourism and hotel business instead of harvesting and trading forest and environmental products.

The broad geographic distribution of *Neopicrorhiza* in the Himalayas implies that this species is an easily accessible economic resource to many rural people (Olsen, 2005; Olsen & Larsen, 2003). The cash income earned by selling high-value medicinal plants, including *Neopicrorhiza* can be an appreciable supplement to local subsistence (Olsen, 2005). Conveniently, in ANCA, the traditional practice of MAP harvesting is legally permitted through the Management Council, formed based on Protected Area Management Regulation, which issues collection and transportation permits for all legal environmental products (HMG/N, 1978). All national parks in Nepal located at high elevation are managed under the Himalayan National Park Regulation (HMG/N, 1978) which also includes a provision to issue harvesting permits to selected MAPs within the park boundary. However, in LNP, permits to collect and trade MAPs have been provided only for *Ophiocordyceps* (personal communication with a conservation officer, 26 July 2018). It therefore stands to reason that, respondents in LNP might be less likely to report commercial collection of *Neopicrorhiza* than respondents in ANCA, where such collection is permitted. Nevertheless, some studies indicate that illegal harvest of MAPs could occur in both regions due to geographical remoteness and lack of regular invigilation mechanisms (Pyakurel et al., 2018; Shrestha, Prasai, Shrestha, Shrestha, & Zhang, 2014; Uprety et al., 2011).

4.2 | Variation in plant density and biomass

Our observations show that the variations in ramet density and rhizome biomass of *Neopicrorhiza* correlate with harvest

intensities and physical habitat variables. The effect of commercial harvesting appeared critical for plant density, rhizome size, and biomass in ANCA. Results indicated that the plant density and biomass are 1.5 times higher in LNP than in ANCA, likely demonstrating that the former is better protected than the latter. Compared to LNP, in ANCA 1.6 times more ramets must be collected to obtain one kilogram (dry weight) of rhizomes. Based on our annual observations, a conservative estimate is that commercial collectors in ANCA collect approx. 364,000 rhizomes (average: 2,136 rhizomes/kg) to achieve their harvest of 170 kg dry weight. This estimate suggests that commercial harvest may have a stronger negative effect on *Neopicrorhiza* populations than other medicinal rhizomatous Himalayan species like *P. kurrooa*, where only 200–500 rhizomes are needed per kg of dry mass (Rai, Prasad, & Sharma, 2000; Uniyal et al., 2011). Given the strong harvest pressure, we suggest an action plan to be prepared for ANCA to mitigate the risk of over-collecting *Neopicrorhiza* rhizomes in this region. A large variation in ramet density between study regions was observed for adult stages with lower density in ANCA than in LNP. Furthermore, a greater proportion of reproductive ramets in LNP than in ANCA may be an additional indication of the heavy collection pressure that affects *Neopicrorhiza* populations in ANCA. Moreover, Ghimire et al. (2005), Ghimire et al. (2008) stated that alpine MAPs, including *Neopicrorhiza*, should be left unharvested for at least 5–10 years to allow sufficient time for individuals to grow and reproduce between the harvests.

Plant density and biomass decreased with increasing elevation, indicating how environmental stresses (e.g., lower temperature, shorter growing time) constrain growth at high elevations (He, Xue, Gao, Wang, & Wu, 2017). However, in LNP reproductive ramet density increased weakly with elevation (Table 2) but, otherwise, the overall effect of elevation was weakly negative in LNP and clearly negative in ANCA. The increase in reproductive ramet density in LNP along the elevation could reflect a reduced harvesting pressure with increasing elevation in combination with a tendency of plants to be more likely to grow to reproductive maturity when growing in less disturbed conditions. However, in ANCA, the negative effect of human disturbance on *Neopicrorhiza* was evident, also for the reproductive stage and regardless of elevation. This finding is consistent with Rusterholz, Kissling, and Baur (2009), who observed that sexual reproduction in the rhizomatous clonal herb *Anemone nemorosa* increased with declining human disturbance.

4.3 | Major habitat determinants

In LNP, the shrub cover and moss/lichen cover showed a positive relationship with *Neopicrorhiza* density ($r = 0.787$, $p < 0.0001$). Hence, it could be suggested that shrub vegetation with substantial moss/lichen ground cover maintains a high moisture level that favors *Neopicrorhiza* populations and leads to increased density. Positive effects of shrub facilitation, such as reduced evapotranspiration, improved thermoregulation, and soil amendment by litter

accumulation and decomposition have been reported in previous studies (e.g., Ballantyne & Pickering, 2015). However, in LNP the shrub cover generally decreased with increasing elevation. Based on the density and biomass estimates in LNP, we suggest low elevation populations (<4,500 m) are likely more resilient to low-intensity harvest, in part due to facilitative effects offered by shrub cover.

However, in ANCA, rock cover showed a positive relationship with plant density (Figure 5a), whereas the relationship between shrub cover and plant density was not significant. Our interviews with MAP collectors revealed that their impression was the opposite, as they believed that, compared to other habitats, shrubland habitats had higher biomass of *Neopicrorhiza* per unit area, which allowed them to collect larger amounts within a given period of time (data not shown). Commercial collectors usually harvest intensively in order to maximize yield while minimizing effort and time. Hence, they prefer to harvest in dense populations where high quantities of rhizomes are available (Ghimire, McKey, & Aumeeruddy-Thomas, 2004). We therefore suggest that the observed higher density of *Neopicrorhiza* in rocky habitats in ANCA may be due to the preference of harvesters for the shrubland habitats at lower elevations.

In our study, grazing showed a positive effect on ramet density except for the reproductive stage (Table 1). Our field observations indicated that *Neopicrorhiza* is unpalatable to herbivores, which could be linked to secondary metabolites, such as terpenoids, cucurbitacin, kutkisterol, and steroids present in the plant (Li et al., 2014). Additionally, in alpine meadows, grazing tends to decrease competition for light without altering functional diversity of foliar traits (Niu et al., 2016). Hence, the removal of species other than *Neopicrorhiza* by grazing reduces competition for light and provides the species with suitable conditions for vegetative regeneration. Indeed, the guerrilla growth form of *Neopicrorhiza* allows it to multiply as soon as it finds open space (Ghimire et al., 2005; Humphrey & Pyke, 1998).

Clonal growth is a successful strategy for persistence of plant populations in stressful environment like those of the Himalayas (Dvorský et al., 2013, 2015; Herben, Šerá, & Klimešová, 2014). Based on the parameter estimates obtained for juvenile and adult vegetative ramet densities it appears that for given levels of harvest and elevation, densities were higher in LNP than ANCA (Table 2). Ghimire et al. (2005) also found higher values of *Neopicrorhiza* density at low levels of harvesting. The plagiotropic growth of the plant enhances its ability to adapt to extreme environmental conditions where the competition from orthotropic plants is less important (Ghimire et al., 2005; Katayama et al., 2015). The ramets are only partly autonomous, which enables them to benefit from nutrients (nitrogen, phosphorus, potash, and urea) and photosynthates (sugars, amino acids, and hormones) made available by already existing ramets of the same genet (Lei, 2010). Previous studies (Evette et al., 2009; Johansen, Wehn, & Hovstad, 2016; Lei, 2010) found that the budding potential and ramet production of clonal herbs in disturbed environments is higher than in undisturbed environments. Consequently, the clonal growth and fugitive establishment of *Neopicrorhiza* could partly remediate the impact of low-intensity harvest in disturbed populations.

5 | CONCLUSION

The present study provides baseline evidence on plant utilization systems, population differentiation, and habitat conditions of *Neopicrorhiza* across a conservation gradient. Both plant density and biomass indicate that a low harvest intensity had no adverse effect on the populations, although quantification of the optimum level of harvest remains to be done and is therefore an aim of ongoing research. Market-driven harvesting and premature exploitation are the most important issues for long-term management. The high number of harvesters in the open-access region appeared to have a strong negative impact on *Neopicrorhiza* populations, whereas in the protected region such clear effects were not observed. Shrubland habitats exhibited high plant densities in the protected region (LNP), but in the open-access region (ANCA), heavy exploitation seemed to occur in such habitats and so, a higher plant density occurred in rocky areas instead. To ensure that the number of reproductive individuals left in each harvested population is sufficient to maintain population viability, we propose regulating harvest such as by introducing rotational harvest systems and in situ management of *Neopicrorhiza* populations. Additionally, we suggest regulating management through implementing community monitoring of harvest, involving local users, collectors, and park officials. Finally, we recommend analyzing the effects of harvest on populations dynamics to estimate suitable limits of annual harvest and develop strategies for sustainable management.

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CONFLICT OF INTEREST

The authors declare that no competing interests exist.

AUTHOR CONTRIBUTIONS

MRP and SKG designed the experiment; MRP collected data; MRP, HM, BBS, and SKG analyzed the data and wrote the manuscript.

DATA ACCESSIBILITY

Data used for ecological analysis are presented in the paper and the supplementary material. Data that identify human participants in the

study will not be made publicly available. However, such data can be made available upon request to the authors.

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