Impacts of Invasive Alien Plant Species on Diversity of Soil Macroinvertebrates in Parsa National Park, Nepal

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Declaration

I hereby declare that the work presented in this dissertation "Impacts of invasive alien plant species on the diversity of soil macroinvertebrates in Parsa National Park, Nepal" has been done by myself, and has not been submitted elsewhere for the award of any degree. All sources of information have been specifically acknowledged by reference to the author(s) or institution(s).

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Recommendation

This is to recommend that the dissertation entitled "Impacts of invasive alien plant species on diversity of soil macroinvertebrates in Parsa National Park, Nepal" has been carried out by Chandu Paneru for the partial fulfillment of master's degree of Science in Zoology with a special paper Ecology and environment. This is her original work and has been carried out under my supervision. To the best of my knowledge, this dissertation work has not been submitted for any other degree in any institution.

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Letter of approval

On the recommendation of supervisor "Associate Prof. Dr. Hari Prasad Sharma" this dissertation submitted by Chandu Paneru entitled "Impacts of invasive alien plant species on the diversity of soil macroinvertebrates in Parsa National Park, Nepal" is approved for the examination in partial fulfillment of the requirements for master's degree of Science in Zoology with special paper Ecology and Environment.

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Abstract

Soil macroinvertebrates play a critical role in regulating organic matter decomposition, nutrient recycling, and infiltration. The change in ecosystem and soil quality around them determines their abundance and diversity. Although it is widely assumed that their composition is influenced by the presence of invasive alien plant species (IAPS), their effects on macroinvertebrates remain little known. Therefore, we studied the ecological impact of two widespread IAPS (*Lantana camara* and *Mesosphaerum suaveolens*) of Parsa National Park (PNP), Nepal on macroinvertebrates, and soil. A total of 45 pairs of invaded and non-invaded plots (5 m × 5 m) were sampled for each IAPS within the Park. Three sites were selected within the park for each of these IAPS. Soil macroinvertebrates were collected using the stainless-steel soil corer method from each plot. Soil samples were also collected from each quadrat to determine soil pH, carbon and nitrogen. A total of 77 species, 70 genera, and 42 families under 15 orders of the phyla Annelida and Arthropoda were recorded. The invaded and non-invaded habitat were compared by calculating abundance, species richness and species diversity. There was no significant difference between abundance, species richness and diversity of macroinvertebrates in the invaded and non-invaded area. However, total number of macroinvertebrates were found more in IAPS invaded area. The abundance, species richness and diversity of macroinvertebrates were influenced by soil carbon, nitrogen and pH. Carbon and nitrogen had a positive impact whereas pH showed a negative impact. Species like *Delathrum* was only present in *M. suaveolens* invaded area. Sensitive Annelids such as earthworms were only present in non-invaded area. The presence of high amount of litter biomass caused an increase in number of macroinvertebrates in invaded area. The results concluded that invasion by *M. suaveolens* and *L. camara* in Parsa National Park has caused significant alterations in macroinvertebrate community and soil physiochemical properties. Therefore, present study suggests investigating the specific mechanism to identify if invasive species provide better habitat and food resources or had just altered soil chemistry in a way that benefits certain macroinvertebrates.

शोध सारांश

माटोमा पाइने ढाडमा हाड नभएका जीवहरूले जैविक पदार्थको विघटन, पोषक तत्वको पुनः प्रयोग र निष्पन्दनलाई विनियमित गर्न महत्वपूर्ण भूमिका खेलेका छन्। माटोको गुणस्तर र वरपरको पारिस्थितिक पद्धतिमा भएको परिवर्तनले तिनीहरूको प्रशस्तता र विविधता निर्धारण गर्छ। यद्यपि विश्वमा तिनीहरूको संरचना आयातित मिचाहा प्रजातिहरूको (IAPS) फैलावटबाट प्रभावित छ भन्ने मान्यता हुँदै आए पनि यसको प्रभाव ढाडमा हाड नभएका जीवहरूमा थोरै ज्ञात छ। तसर्थ हामी ले पर्सा राष्ट्रिय निकुञ्ज (PNP) अन्तर्गत त्यहाँ फैलिएका दुई व्यापक आयातित मिचाहा प्रजातिहरूको (*Lanata camara* and *Mesosphaerum suaveolens*) असर माटोमा रहेका ढाडमा हाड नभएका जीवहरू सँगको प्रचुरता र माटो का विभिन्न मापदण्डहरु (pH, कार्बन र नाइट्रोजन) सँगको सम्बन्ध अध्ययन गर्यौ। हामीले पार्कभित्र दुवै मिचाहा प्रजातिहरूका लागि प्रत्येक ४५ जोडी मिचाहा प्रजातिहरू भएका र नभएका प्लटहरू ५ m × ५ m) नमुना लिएका थियौं। साथै प्रत्येक मिचाहा प्रजातिहरूका लागि पार्क भित्र तीन(तीनवटा स्थानहरू चयन गरिएको थियो। Stainless-stell soil corer विधि प्रयोग गरेर माटोमा रहेका ढाडमा हाड नभएका जीवहरू सङ्कलन गरिएको थियो। अध्ययन अवधि भरमा ढाडमा हाड नभएका जीवहरूका ४२ परिवारहरू (Families) र १५ अडरहरूबाट जम्मा ७० जाति सहित ७७ प्रजातिहरू सङ्कलन गरिएको थियो। माटोको प्रचुरता, प्रजाति समृद्धि र प्रजाति विविधता गणना गरेर मिचाहा प्रजातिहरू भएका र नभएका प्लटहरू तुलना गरिएको थियो। मिचाहा प्रजातिहरू भएका र नभएका प्लटहरूमा प्रचुरता, प्रजाति समृद्धि र ढाडमा हाड नभएका जीवहरूको प्रजाति विविधताबीच कुनै महत्त्वपूर्ण भिन्नता थिएन। यद्यपि मिचाहा प्रजातिहरू भएका प्लटहरूमा ढाडमा हाड नभएका जीवहरू प्रचुर मात्रामा पाइएका थिए। ढाडमा हाड नभएका जीवहरूको प्रचुरता, प्रजाति समृद्धि र प्रजाति विविधता माटोमा रहेको कार्बन, नाइट्रोजन र pH द्वारा प्रभावित थियो। कार्बन र नाइट्रोजनले सकारात्मक प्रभाव पारेको थियो। ज्बकि pH ले नकारात्मक प्रभाव देखायो। *Delathrum* जस्ता प्रजाति *M. suaveolens* उपस्थित प्लटहरूमा मात्रै पाइएको थियो। गँड्यौला जस्ता संवेदनशील एनेलिडहरू मिचाहा प्रजातिहरू नभएका प्लटहरूमा मात्र उपस्थित थिए। लिटर बायोमासको उच्च मात्राको उपस्थितिले मिचाहा प्रजातिहरू भएका प्लटहरूमा ढाडमा हाड नभएका जीवहरूको संख्यामा वृद्धि भएको छ। यस नतिजाले पर्सा राष्ट्रिय निकुञ्जमा मिचाहा प्रजातिहरूको आक्रमणले ढाडमा हाड नभएका जीवहरूको समुदाय र माटोको भौतिक र रसायनिक गुणहरूमा महत्वपूर्ण परिवर्तन ल्याएको देखाउँछ। तसर्थ, वर्तमान अध्ययनले मिचाहा प्रजातिहरूले राम्रो बासस्थान प्रदान गर्दछ वा निश्चित ढाडमा हाड नभएका जीवहरूलाई फाइदा पुऱ्याउने तरिकामा माटोको रसायन परिवर्तन गरेको छ कि छैन भनेर पहिचान गर्नको लागि विशेष संयन्त्रको अनुसन्धान गर्न सुझाव दिन्छ।

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List of abbreviations

Abbreviated form	Details of abbreviations
IAPS	Invasive Alien Plant Species
PNP	Parsa National Park
BZ	Buffer Zone
GPS	Geographical Positioning System
PA	Protected Area
C	Carbon
N	Nitrogen

1. Introduction

1.1Background

Soil is home to diverse biological communities, potentially estimated for up to 40% of all species on earth (Orgiazzi et al., 2016). It is a species-rich environment integral to terrestrial ecosystems (Lavelle et al., 2006), playing a crucial role in organic matter and nutrient recycling (Snyder, 2008). Soil invertebrates, which inhabit both the soil surface and subsurface, are categorized by size into microfauna (< 2 mm), mesofauna (0.2–2 mm), and macrofauna (>2 mm) (Lavelle et al., 2016). Macroinvertebrates, are those larger than 2 mm and visible to the naked eye, exhibit broad ecological roles and significantly influence soil processes (Levelle et al., 2022). Due to their extensive distribution and activity in both temperate and tropical environments (Lavelle, 1997), they are often termed soil engineers (Jones et al., 1994). This group includes Annelids, Mollusks, Arthropods, Arachnids, Crustaceans, Odonats (mayflies, dragonflies, and damselflies), stone flies, true bugs, beetles, caddisflies, and true flies (Lavelle et al., 2006). Based on their ecological role, macroinvertebrates are classified as shredders, predators, herbivores, and fungus feeders (Fahad et al., 2022).

Soil macroinvertebrates either continually or partially impede (at least one of their developmental stages) on soil (Lavelle et al., 1996; 1997; 2006; Pereira et al., 2017). Species like millipedes (Fujimaki et al., 2010), ants (Cammeraat & Risch, 2008), earthworms (Blouin et al., 2013a), termites (Jouquet et al., 2011), and beetle larvae (Nicholas et al., 2008) significantly impact their physical environments. These organisms play an important role in water supply, nutrient cycling, primary production, soil formation, and serve as indicators of soil quality (Lavelle et al., 2006). Their activities facilitate the release of essential nutrients necessary for plant growth, enhance soil purification, and increase the soil's water storage capacity (Fahad et al., 2022).

The physio-chemical properties of the soil, including its porosity, pH, cation exchange capacity, organic matter content, moisture content, and soil temperature, are critical determinants of soil health (Chen et al., 2016; Kekane et al., 2019). These parameters influence the composition and abundance of soil invertebrates, causing both qualitative and quantitative changes in the soil invertebrate communities (Cortel et al., 1999). Macrofaunal taxa like Chilopoda, Diplopoda, Coleoptera, Hemiptera, Formicidae, Isopoda, and Aranea are affected by different land use systems (Pereira et al., 2017; Yin et al., 2019). Soil

arthropods, such as collembola, are sensitive to disturbance. In addition, species like spiders and ground beetles can provide valuable information regarding soil-metal pollution (Dallinger et al., 1992; Paoletti et al., 1999). It is known that soil properties can be significantly altered by exotic plant species or biological invasions (Cortet et al., 1999).

Biological invasion is one of the top five most detrimental consequences of human activity on planetary changes (IPBES, 2019). Factors such as increase in global trade, changes in land use, human mobility, and various tourism-related activities have contributed to the global rise in alien and invasive species (Seebens et al., 2017; Pathak et al., 2021; Hulme 2021). Invasive alien species (IAS) are a subset of alien species including both plants and animals, becoming established in new areas with detrimental effects on biodiversity, and regional ecosystems (IPBES, 2023). The proliferation of IAS has led to a decline in biodiversity, modification of the evolutionary paths, alteration to ecological processes, a decrease in ecosystem services, and increase in economic costs (Davis 2009; Diagne et al. 2021; IPBES, 2023). The promotion of tourism has significantly increased human activity in protected regions, resulting in the rapid expansion of invasive alien plant species (IAPS) into these areas (Pathak et al., 2021). Interactions between IAPS, native plants, and herbivores determine the success of these species in their new environments (Levine et al., 2003). Terrestrial habitat degradation caused by IAPS is an urgent issue requiring the attention of ecologists and conservationists globally (Shrestha, 2016). Among all established foreign plants in the globe, at least 1061 are recognized to be invasive (IPBES, 2023). The number of IAPS is expected to grow, especially in protected areas and most natural ecosystems (IPBES, 2023), causing a negative influence on biodiversity and native species in the invaded ecosystems (Hulme et al., 2013; Early et al., 2016).

In Nepal, the species richness and distribution patterns of IAPS vary with altitude and phytogeographic regions (Shrestha, 2016). Most IAPS are prevalent in the Tarai and Siwalik, regions, which feature tropical to subtropical climates similar to their native habitats (Tiwari et al., 2005; Shrestha et al., 2017). A total of 30 species are identified as IAPS in Nepal (Shrestha et al., 2024), including siam weed (*Chromolaena odorata),* water hyacinth (*Pontederia crassipes),* kirne kada (*Lantana camara*), cogon grass (*Imperata cylindrica*) and mile-a-minute (*Mikania micrantha),* which also listed among the 100 worst IAPS globally (Lowe et al., 2000). The colonization of these IAPS poses a significant threat to Nepal's protected areas, with *C. odorata*, *M. micrantha*, *L. camara*, and congress grass (*Parthenium hysterophorus)* being particularly problematic in terrestrial ecosystems

(Bhuju et al., 2013). *L*antana *camara*, for instance, demonstrates a highly competitive ability against native plant species (Raphela & Duffy, 2022). It releases allelochemicals like Phenolic acid, Triterpene, and Flavonoids into the soil leading to phytotoxic activity that reduces the seedling recruitment of native plants (Ghisalberti, 2000; Verdeguer, 2009). Additionally, *L. camara* can alter the activity and structure of soil microbial communities, as bacteria are susceptible to environmental change, which can subsequently impact the entire ecosystem (Kourtev et al., 2002; Gaggini et al., 2018).

Similarly, the invasion of *M. suaveolens* has a detrimental impact on plant diversity (Sharma et al., 2009; 2017) and local vegetation (Sharma et al., 2017), primarily due to its allelopathic chemicals that are particularly harmful to native flora. This IAPS also repels several Coleoptera species, including wheat weevil (*Sitophilus granaries)* and cowpea weevil (*Callosobruchus maculatus*), lesser grain borer (*Rhyzopertha dominica),* rice weevil *(Sitophilus oryzae).* Furthmore, the presence of *M. suaveolens* has been observed to repel various Diptera species (Rahmatullah et al., 2012).

The understanding of the invasion of IAPS and their impacts on soil physicochemical parameters, as well as macroinvertebrate abundance and diversity remains limited in the context of Nepal. Although contemporary studies on IAPS and their distribution have been conducted (Bhatta et al., 2020; Chaudhary et al., 2020; Chand & Sharma, 2023), few have specifically examine the effects of IAPS on macroinvertebrates (Pysek et al., 2012; Zhang, 2019; Raphela et al., 2022). The primary gap of current research is the lack of data on the interaction between IAPS and soil macroinvertebrates in Nepal. This deficiency hampers effective management and impact assessment efforts.

Species like *L. camara* and *M. suaveolens* are spreading rapidly in the Parsa National Park (PNP) (Chaudhary et al., 2022), adversely affecting native flora and fauna. Therefore, this study aimed to investigate the effects of these two IAPS on soil physicochemical parameters, and macroinvertebrates diversity in PNP. The outcomes of this research can be used for conservationists, policymakers, and managers to develop policies to control and manage IAPS in PNP.

1.2 Statement of the problem

The IAPS is recognized as one of the most significant threats to global biodiversity, impacting ecosystem structure, function, and native species (IPBES, 2019; 2023; Shrestha et al., 2024). The PNP, a biodiverse region in Nepal, is facing the encroachment of IAPS,

which could potentially disrupt ecological balance (Chaudhary et al., 2020). Most studies on the impact of IAPS have focused on native plant species and large mammals (Bhattrai et al., 2014; Darji et al., 2021; Bhatta et al., 2022; Adhikari et al., 2022; Chand & Sharma, 2023; Paudel et. al 2023). However, there is limited research on how these IAPS affect macroinvertebrates in Nepal (Pandey et al., 2020; Basaula et al., 2022; Pandey, 2024). The primary gap in current research is the lack of data on the interaction of IAPS and soil macroinvertebrates in the case of Nepal. This deficiency creates problems for effective management and accurate impact assessment. Species like *L. camara* and *M. suaveolens* are spreading rapidly in the case of PNP (Chaudhary et al., 2020) causing adverse impacts on native flora and faunas. Therefore, this study provided baseline data on the diversity of macroinvertebrates in the IAPS Invaded and non-invaded area of PNP.

1.3 Research objectives

1.3.1 General objective

The general objective of this study was to investigate the impacts of IAPS on the diversity of macroinvertebrates in Parsa National Park, Nepal.

1.3.2 Specific objectives

The study had following specific objectives:

I. To compare the diversity of macroinvertebrates in IAPS invaded and non-invaded area.
II. To assess the effects of soil parameters on macroinvertebrates in IAPS invaded and non-invaded area

1.4 Research Hypothesis

The following hypothesis had been formulated for the study.

- H_0: IAPS reduces abundance, species richness and diversity of soil macroinvertebrates.
- H_1: IAPS alters the soil characteristics in the areas it invades.

1.5 Significance of study

Many communities depend on the resources provided by national parks for their livelihoods, including ecotourism and ecosystem services. However, the protected areas of

the world have been infested by IAPS (Foxcroft et al., 2017; Moodley et al., 2022) including Nepal too (Thapa & Maharjan, 2014; Chaudhary et al., 2020; Bhatta et al., 2020). Though the extent of plant invasion was predicted to be limited due to more wilderness inside the park, recent studies have shown that they can seriously influence the ecosystem (Foxcroft et al., 2017). Likewise, IAPS can have a negative impact on native species (Shrestha et al., 2015) by altering their habitat structure and reducing biodiversity (Kohli et al., 2006; Shrestha et al., 2016; Shrestha & Shrestha, 2021). This in turn inhibits the health hazard of humans due to their allelopathic properties (Patel, 2011) and influences the soil parameters and microorganisms (Timsina et al., 2011).

The diversity and species composition of soil macroinvertebrates are integral to terrestrial ecosystems. They play a crucial role in biological engineering, water supply, nutrient cycling, primary production, soil formation, and climate regulation (Lavelle et al., 2006). But the understanding of the invasion of IAPS and its impacts on physicochemical parameters of soil, and macroinvertebrate abundance and diversity remains limited. Though studies on IAPS and their distribution are carried out nowadays in Nepal (Bhatta et al., 2020; Chaudhary et al., 2020), there are countable studies that have looked at the effect of IAPS on macroinvertebrates (Pysek et al., 2012; Zhang, 2019, Raphella et al., 2022). The main gap is unavailability of data regarding IAPS interaction with soil macroinvertebrates in case of Nepal. Little knowledge is available on the association of IAPS with macroinvertebrates which creates a problem for proper management and identifying impacts due to data deficiency. IAPS like *L. camara* and *M. suaveolens* are spreading rapidly in the case of PNP (Chaudhary et al., 2020) causing adverse impacts on native flora and faunas.

Therefore, this study aimed to investigate the effects of *L. camara* and *M. suaveolens* on the physicochemical parameters, as well as the diversity of macroinvertebrates in PNP. The output of this research could be fruitful for conservationists, policymakers, and managers in developing policies to control and management of IAPS as well as the conservation of biodiversity in PNP. The findings of this study could serve as the baseline data for further investigation about the invasion of these IAPS and their impacts on macroinvertebrate diversity.

1.6 Limitations of the study

The study has the following limitations:

- The major limitation of the study was high risk of wildlife in both Protected Areas. Due to this reason, the planned sampling sites were affected and unable to conduct sampling in the area where there is high risk of wildlife.
- Data collection was limited to a specific period, which does not represent the seasonal variations in macroinvertebrate populations. Different seasonal variations may affect the associated macroinvertebrates and soil characteristics.
- Identification of all macroinvertebrates up to species level was not done.

2. Literature review

2.1 Diversity of macroinvertebrates

The occurrences and assemblages of soil fauna differ in all ecosystems. Woody plants offer high surface litter, the best nutrient cycling, and a dense root system that creates a stable microhabitat (Scherber et al., 2010; Bayranvand et al., 2017). Shrublands provide qualitatively distinct litter, while in grasslands, the root system is the only source of soil nutrients (Cuchta, 2020). Additionally, forest regions may exhibit seasonal variations in biodiversity due to climatic change (Martin-Chave et al., 2019). More diversity in the litter assures more variation in the fauna, but not necessarily abundance (Paul et al., 2011; Zagatto et al., 2019a). Soil fauna have a specific preference for litter as a food source (Warren & Zou, 2002). For example, earthworms prefer fresh deciduous leaf litter (Paoletti, 1999), while mites, springtails, and Enchytraeid worms prefer coniferous forests with flourishing fungi (Cuchta, 2020).

In mixed plantations, the most palatable litter broke down more quickly by diverse groups whereas litter with low nutritional values is preferred only when other resources are scarce (Guille et al., 2019; Tresch et al., 2019). The litter mass only affects mesofauna; however, for macrofauna, other parameters such as plant covering and soil organic carbon have to be considered (Wu & Wang, 2019). Likewise, a healthy surface litter cover supports more varied populations of litter transformers, such as Myriapoda, Isopoda, and Arachnida. However, in herbaceous ecosystems, earthworms and Coleoptera can become dominant (Lavelle et al., 2022). Species such as earthworms are more prevalent in younger soils, termites in older soils, and ants are prevalent in both the youngest and oldest soils (Orgiazzi et al., 2016).

2.2 Relationship of macroinvertebrates with environmental variables

The diverse environmental factors, such as plant cover, temperature, rainfall, latitude, and soil texture, each have a unique impact on the overall variance (Lavelle et al., 1993) and community assemblages of soil invertebrates (Zagatto et al., 2017; Yang et al., 2020; Uhey et al., 2020). The distribution and density of macroinvertebrates under various land coverings are primarily influenced by temperature (soil and air) and moisture (Zagatto et al., 2019b; Kooch & Noghre, 2020). Studies on the correlation of soil fauna with physical parameters have shown that lower temperature or high moisture decreases their abundance

in soil but not necessarily in litter. On the other hand, high temperature and humidity encourage diversity and density in comparison to drier locations because high moisture facilitates these faunae (Laiho et al., 2001, Gonzalez & Seastedt, 2001; Zagatto et al., 2019a; 2020). This correlation also explains pronounced seasonal variation where higher abundance is seen in autumn and spring and least in winter (Zagatto et al., 2017, Yin et al., 2018; 2019).

Additionally, soil characteristics like lower porosity, bulk density, and higher salinity affect the abundance of mesofauna inversely (Machado et al., 2019; Zagatto et al., 2019b; Kooch & Noghre, 2020) as the majority of fauna live in topsoil (Lee & Foster, 1991, Yin et al., 2018; 2019). In contrast to this, the response of macrofauna was not found significant, except for Chilopoda, for they have relatively higher tolerance and diversity in adaptation (Yin et al., 2018; 2019).

Research has shown that there were notable differences in the community composition between anthropogenically impacted systems and natural systems (such as preserved areas) (Koehler, 1992; Baretta et al., 2007; Pereira et al., 2017; Santos et al., 2018; Zagatto et al., 2019a; 2020). Additionally, mesofaunas such as mites and springtails are found to be greatly affected in different land use systems (Yin et al., 2019; Van Langevelde et al., 2020). However, macrofaunal taxa like Chilopoda, Diplopoda, Coleoptera, Hemiptera, Formicidae, Isopoda, and Aranea have been reported to be affected as well (Pereira et al., 2017; Yin et al., 2019).

2.3 Impacts of *L. camara* on soil parameters and macroinvertebrates

After the introduction of IAPS in a healthy ecosystem, they disrupt the fungal mutualistic associations with native plants by changing the nutrient dynamics and interrupting the food webs of soil (Ehrenfeld et al., 2005). As a result, the richness and abundance of soil biota increases due to the invasion of exotic species (Pysek et al., 2012). The invasive species produce more leaf litter with a lower carbon: nitrogen ratio compared to native species which increases the availability of carbon in the soil and allows the establishment of a diverse and abundant soil microbe's community (Zhang, 2019). Introducing IAPS can alter the activity and structure of soil microbe communities (Kourtev et al., 2002) because bacteria are sensitive to changes in the environment resulting in changes in the whole ecosystem (Gaggini et al., 2018). When the IAPS once replace native plants, they can modify fungal communities and increase functional similarities of fungal pathogens even

at low plant density which may limit negative soil effects on invasive plants (Wang et al., 2015).

Lantana camara has a high nutrition extraction efficiency compared to other shrubs and herbs, potentially leading to increased competition for nutrition in the ecosystem (Bhatt et al., 1994, Ruwanza & Shackleton, 2016; Vilizzi et al., 2022). The sites invaded by *L. camara* have a high total organic carbon, organic phosphorus, soil moisture, and decrease in pH (Vilizzi et al., 2022) and are repellent because they can retain productivity in degraded land, translocate nutrients from senescing foliage back into the soil (Bhatt et al., 1994).

Though most studies support the idea that an IAPS-invaded area favors more microbiota than a non-invaded area, few studies have mentioned that an invaded area supports fewer invertebrates.

Lantana camara vegetation supports fewer invertebrates due to its low biomass and less morphospecies richness than grass-dominated vegetation (Raphela et al., 2022). However, the extensive cover by one plant species, like *L. camara*, has been associated with unsuitable conditions for detritus invertebrate colonization (Palmer et al., 2004). The spread of *L. camara* also negatively influences the productivity of riparian habitats in terms of invertebrate biomass (Gerber et al., 2008; Raphela & Duffy, 2022).

2.4 Impacts of *M. suaveolens* on soil parameters and macroinvertebrates

The decrease in native species diversity in *M. suaveolens* invaded area exhibited lower moisture content, pH, and temperature, due to its shade cover (Afreen et al., 2018). Whereas soil ammonium-nitrogen, total inorganic-nitrogen, and nitrogen mineralization registered higher values for invaded areas (Sharma et al., 2009; 2017; Aboh, 2017; Afreen et al., 2018). The alteration of soil chemical components in the invaded area is due to a high amount of above-ground biomass (Kourtev et al., 1998; Koutika et al., 2007; Liao et al., 2008).

Mesosphaerum suaveolens is an important source of essential oils, alkaloids, flavonoids, phenols, saponins, triterpenes, and sterols (Edeoga et al., 2006, Sharma et al., 2012). The essential oil present in this IAPS can repel mosquito species like the African malaria mosquito (*Anopheles gambiae)* and yellow-fever mosquito (*Aedes aegypti)* (Seyoum et al., 2002; Rahmatullah et al., 2012). Likewise, insects of Coleoptera like wheat weevil

(*Sitophilus granaries)* and cowpea weevil (*Callosobruchus maculatus*), lesser grain borer (*Rhyzopertha dominica),* rice weevil *(Sitophilus oryzae),* and red flour beetle (*Tribolium castaneum)* are repelled. Also, the species of arachnids of public health and economic interest like cayenne tick (*Amblyomma cajennense)* and castor bean tick (*Ixodes ricinus)* are found to be repelled by *M. suaveolens* (Almeida-Bezerra et al., 2022). It means these macrofauna's avoid *M. suaveolens* presence area. A recent study found that it is used as biopesticides by indigenous people of Plateau State, Nigeria (Ali et al., 2022).

2.5 Research gap

The invasion of *L. camara* (70-1715 m asl) and *M. suaveolens* (75-1065 m asl) has already spread in vast areas of Nepal (Shrestha & Shrestha, 2021). Ecological niche modeling studies suggest the probability of further expansion of the weed in Nepal with climate change in the future (Shrestha et al., 2019). Various tourism-related activities have resulted in the rapid expansion of IAPS in protected areas like Chitwan National Park (CNP) (Murphey et al., 2013). Numerous studies have been conducted to understand the impacts of IAPS on native flora and large fauna (Bhatta et al., 2020; Chaudhary et al., 2020; Darji et al., 2021; Chand & Shrestha, 2023) while fewer studies to understand the impacts on lower organisms like macroinvertebrates (Pysek et al., 2012; Zhang, 2019; Raphela et al., 2022). However, limited studies are conducted on macroinvertebrates impact in case of Nepal (Pandey et al., 2020; Basaula et al., 2022, Pandey 2024). Macro-invertebrates serve a crucial role in the biological process of energy transfer to consumers through detritus. However, little is known regarding *L. camara* and *M. suaveolens* impacts on the diversity of macroinvertebrates and soil parameters in PNP. Therefore, the present study was investigated to provide baseline information on the effects of these IAPS on the physicochemical parameters and diversity of soil macroinvertebrates in PNP.

3. Materials and methods

3.1 Study Area

The study was carried out in Thori Municipality Ward No. 5 Subarnapur (27º 22' N and 84º 43' E), Bijayabasti ward No. 4 (27º 51' N and 84º 41' E), Jirabhawani Rural Municipality Ward No. 1 Shanti tole (27º 13' N and 84º 43' E), Hetauda Submetropolitian City Ward No. 13 Thulothali Lamitar sector (27º 24' N and 84º 58' E) and Manahari Rural Municipality Ward No. 6 Paratappur (27º 31' N and 84º 46' E) of Parsa National Park (PNP; 27º 15' to 27º 33' N and 84º 41' to 84º 58' E). The PNP comprises three districts i.e. Makawanpur, Parsa, and Bara which lie in the lowland of Tarai, Nepal with an elevation of 100 m - 950 m above sea level (DNPWC, 2019). It was established in 1984 A.D and later updated to National Park in 2017. PNP covers 627.39 km^2 of core area and 285.30 km^2 of buffer zone. (DNPWC, 2018; Thapa, 2016). From North to South, the park can be roughly split into the Churia (Siwalik), Bhabar-Tarai, and inner-Tarai topographic regions (DNPWC, 2019). The soil is made up of erodible conglomerates and gravel. Water is scarce in this park because of the highly porous foothills (Bhuju et al., 2007). PNP lies in the humid subtropical climatic zone offering a hot summer (May-Jul), rainy season (Jul-Sep), cold winter (Nov-Jan), and pleasant spring (Feb-Apr). Between October and December, there are clear skies as well as pleasant temperatures. The temperature may range from 0ºC (night) - 40ºC (DNPWC, 2019). The Park consists of a mean minimum temperature of 7°C to a mean maximum temperature of 39°C. 83% of the total precipitation occurs mainly from June to September (PNP, 2018). Sand extraction, shifting cropland, and domestic cattle grazing are relatively high in PNP and BZ (CHEC Nepal, 2012).

3.1.1 Flora and Fauna

The PNP has eight types of ecosystems and two types of forest vegetation (Bhuju et al., 2007) dominated by Satisal (*Dalbergia latifolia* and its associated species like sal (*Shorea robusta),* Kumkum (*Mallotus philippensis*), golden shower (*Cassia fistula*), ban khuwalu (*Lisea monopetala*), Indian rosewood (*Dalbergia sissoo*) and khair (*Sen*egalia *catecehu*) (DNPWC, 2019). Likewise, 37 species of mammals, more than 500 species of birds, 13 species of reptiles, 31 species of insects, eight species of Pisces, and 336 species of plants make up the Park's overall biodiversity (PNP, 2018; Bhuju et al., 2007; DNPWC, 2019).

The park supports good populations of various endangered species which include Asian elephant, tiger (*Panthera tigris tigris*), shaggy-coated sloth bear (*Melurus ursinus*), and leopard (*Panthera pardus*), nilgai (*Boselaphus tragocamelus*), sambar (*Rusa unicolor*),

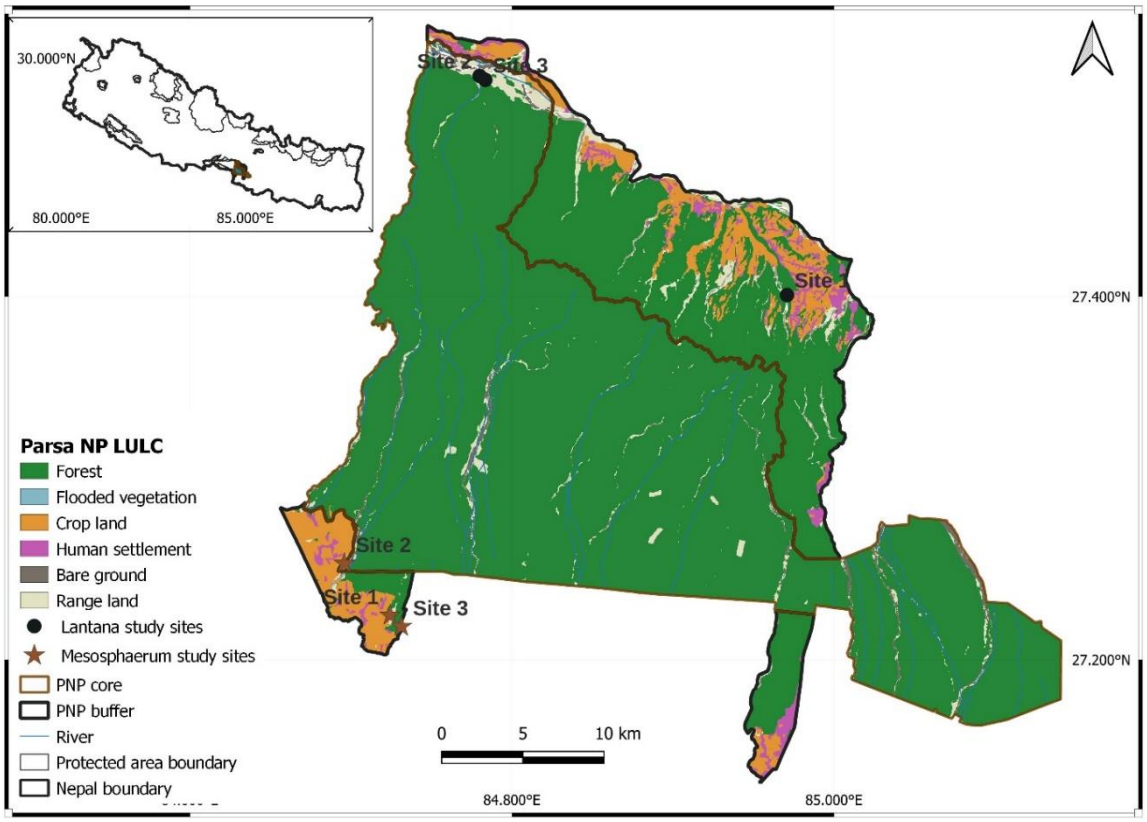

Figure 1: Land use land cover map showing the location of survey sites in PNP, Nepal. The map in the inset shows protected areas of Nepal

barking deer (*Muntiacus vaginalis)*, striped hyaena (*Hyaena hyaena*), jungle cat (*Felis chaus)*, and common palm civet *(Paradoxurus hermaphroditus)*, etc. are also found within the park (DNPWC, 2019). Bird species like white-breasted kingfisher (*Halcyon smyrnensis*), paradise flycatcher (*Terpsiphone paradise*), large racquet-tailed drongo (*Dicrurus paradiseus*), golden-backed woodpecker (*Dinopium benghalense*) etc. are found. The reserve is also famous for reptiles and different kinds of snakes which include common cobra (*Ophiophagus hannah*), banded karit (*Bungarus fasciatus*), python (*Python molurus*), and king cobra (*Ophiophagus hannah*) (DNPWC, 2019). PNP is a part of Terai Arc Landscape in lowland Nepal. It has a transboundary linkage with the Valmiki Tiger Reserve in India through the Shikaribas biological corridor and is also linked to Chitwan National Park towards the west (DNPWC, 2018). Altogether, 14 IAPS have been recorded in the PNP (Chaudhary et al., 2020) including the world's worst IAPS such as kirne Kada (*Lantana camara)*, lahare banmara *(Mikania micarantha)*, and seto banmara (*Chromolana*

odorata). The increase in abundance of these IAPS along with ban tulsi (*Mesosphaerum suaveolens)*, and santo tapre (*Senna tora),* has been found inside the park and near human settlement (Chaudhary et al., 2020).

3.2 Materials and methods

3.2.1 Materials

GPS, sieve, forceps, aspirator, white sheet, 75% alcohol, vials, tape, rope, brush, soil corer, datasheets, and pencil

3.2.2 Methods

3.2.2.1 Sampling method

The field data was collected during November 2–11, 2023. Suitable sites for the IAPS Invaded and non-invaded area were identified after the consultation meeting with park officials and local people. *L. Camara* and *M. suaveolens* were the most widespread and abundant species in the buffer zone near human settlements. Therefore, the impact assessment of *L. camara* and *M. suaveolens* on macroinvertebrates was conducted in PNP.

For this study, three sites were chosen for each of these IAPS within invaded and non-invaded areas. The plots were defined as invaded if the coverage of IAPS was > 50%, while non-invaded plots were those where invasion of IAPS was < 10% or absent. A total of 90 plots (45 plots in each invaded and non-invaded area) were established in PNP. Among these, 45 pair plots were established for *M. suaveolens* in Subarnapur, Bijayabasti, and Santi tole of Ram Bhuri Bhata sector. Likewise, 15-pair plots for *L. camara* were established in the Thulothali area and the remaining 30 pairs were established in Pratappur of Lamitar sector due to the widespread presence of *L. camara* and high security from wildlife. In each habitat, 5m×5m parallel plots were established. The distance between these plots in each site was 5 m apart to reduce the possible differences in the macroinvertebrate community. The coordinates of each plot were recorded using GPS.

3.2.2.2 Macroinvertebrate survey

Soil samples were collected from each 5 m X 5 m plot in which five replicates within the plot were chosen (four at the corner and one at the center) for both IAPS presence and absence habitat. A stainless still soil corer method was used to collect soil macroinvertebrates where a stainless-steel soil corer of diameter 5 cm and depth 10 cm

deep was used. Thus, collected soil was sieved using a standard sieve size 9.5 mm and 850 µm on white sheet paper to extract soil macroinvertebrates. Individual macroinvertebrates were separated by forceps and kept in vials containing 75% ethanol. Species were kept separately according to the plot so that the tissue of the species would remain hydrated and prevent bacterial growth. All the species were labeled with plot no. and date which helped in providing ground correct information about each plot.

3.2.2.3 Identification and Preservation

Collected macroinvertebrates were brought to the laboratory of Central Department of Zoology, sorted based on morphospecies richness, identified and photographed by Sony Cyber-shot DSC-W710 under the stereomicroscope using measuring scale. Photographs were taken using a Samsung S8 smart phone. The macroinvertebrates were identified up to possible taxonomic levels with the help of relevant keys (Borror and Delong 1971; Roonwal & Chhotani, 1989; Subedi, 2023; Bonelli, 1810; Liebherr, 1986; Blakemore, 2006; Jukla, 1988; Chaudoir, 1875; Solier, 1834; Edgecombe et al., 2016). Soil macroinvertebrates were kept in a separate vial containing 70% ethyl alcohol and labeled properly with the mention of taxonomic information of species, date and place of collection, and name of collector. These specimens were later deposited in the CDZM TU, Kirtipur, Nepal with specific taxonomic information.

3.2.2.4 Soil sample collection

The soil from the study plot was collected following the methodology of Stohlgren et al (1998). A 100 gm of soil sample was taken from each plot in a Ziplock bag during the macroinvertebrates collection. Soil samples from five replicates within each plot were thoroughly mixed to create a composite sample, which was then labeled to keep plot-wise information. In total, 180 composite soil samples were collected, with 90 samples from invaded plots and 90 from non-invaded. These samples were transported to the laboratory, air-dried and sieved before nutrient analysis. The soil samples were analyzed for Organic Carbon, Nitrogen, and pH (Gupta, 2000).

3.2.2.5 Soil Parameter Analysis

For several soil parameters, laboratory analysis was carried out in Central Department of Botany, Tribhuvan University. Soil pH was determined by method outlined by Gupta

(2006), soil organic carbon by Walkey and Black method (1965) and Nitrogen was determined by micro-Kjeldahl method.

a. Soil pH

The pH of soil was measured with the help of digital pH meter (model-HM-1003). For this, 20 g of dried and finely powdered soil was taken, and 40 ml of water was added to make a 1:2 soil-water ratio. Then the solution was left for 30 minutes and swirled with the help of glass rod. Before using the pH meter, the pH meter was calibrated with buffer solution of 4.0 and 7.0 simultaneously. Then the pH meter was dipped in soil water solution and reading was noted carefully.

b. Soil organic carbon

First of all, 0.5 g air dry sieved soil was taken in 250 ml clean and dry conical flask. Then 5 mL of potassium dichromate was added and shaken it gently. Similarly, 10 ml of concentrated sulphuric acid was added to the soil. After that, the solution in a conical flask was settled down for about 30 minutes and 100 ml of distilled water was added. Again, 5 mL of phosphoric acid was added to it and then 1 ml of diphenylamine indicator was added. Finally, the whole mixture was ready for titration with a burette containing ferrous ammonium sulphate of 0.5 N normality. The titration was carried out till the color was changed from blue violet to greenish color. A blank mixture was also run without soil. The soil sample and all the chemical content were taken half for determination of soil organic carbon due to the soil containing high organic matter.

Percentage organic carbon in soil sample = $0.003 \times \frac{10\ (Blank\ reading - Titration\ reading)}{Blank\ reading \times wt.of\ soil\ (g)} \times 100$

The obtained percentage carbon was multiplied by factor 1.3 to obtain soil organic carbon.

c. Total nitrogen

The Micro-Kjeldahl method, as reported by Black et al., (1965) was used to determine the total soil nitrogen. The micro-Kjeldahl method includes following three steps:

Digestion: A clean and dry Kjeldahl digestion flask was filled with 1.0 g of air-dried and sieved soil. Then 3.5 g of potassium sulphate and 0.4 g of copper sulphate were mixed in a digestion flask containing soil. After that, 6 mL of concentrated sulphuric acid was added carefully with the help of sucker and pipette. Then the flask was shaken gently and placed

for digestion on a pre-heated (40ºC) heating mantle. The temperature was raised to about 300ºC. When the temperature was raised, the color of soil was changed from black to brownish at the end of digestion. The occurrence of a greenish color indicates over-digestion. When the color of soil was changed from black to brownish, the digestion flask was taken off quickly and left to cool for 5-10 minutes. Then 30 ml of distilled water was added to it, and it was ready for distillation process. Similarly, a blank was run without soil sample.

Distillation: The distillation process was carried out manually in Kjeldahl distillation flask. The aliquot was poured into distillation flask and warmed. Then 30 mL of 40% sodium hydroxide (NaOH) was added. Similarly, 20 ml of boric acid indicator was added on to beaker where the condenser nozzle was dipped down on it. Then the temperature was raised to 70ºC, and the boric acid indicator turned green from pink as the distillate started to condense. The distillation process continued until the beaker contained volume of more than 40 mL for titration.

Titration: When the distillation was complete, titration was carried out with 0.1N hydrochloric acid (HCl). Titration reading was noted carefully and HCl volume consumed by blank was also noted. Then the total nitrogen content (%) was determined by using the following formula below:

$$\text{Soil N (\%)} = \frac{14 \times N \times (S - B) \times 100}{M}$$

Where, N = normality of HCl

S = volume of HCl consumed with sample (mL)

B = volume of HCl consumed with blank (mL)

M = mass of soil taken (mg)

3.2 Data analysis

Before analysis, a correlation test was used among the independent variables to identify whether these are highly correlated $|r| > 0.7$ (Figure 2). None of the variables were highly correlated and all were included in the analysis. The abundance and species richness of macroinvertebrates were calculated using Microsoft Excel 2019. The diversity of soil macroinvertebrates in both IAPS invaded and non-invaded plots was quantified by

computing the Shannon -Wiener diversity index (H′) (Shannon & Weaver, 1948) using package vegan (Oksanen et al., 2023). The following formulas were used to calculate the given diversity indices of macroinvertebrates.

Shannon Wiener's diversity index (H') = $-\sum\left(\frac{ni}{N}\right)\ln(\frac{ni}{N})$

Where,

ni = Importance values for each species is the sum of individuals in each species, the abundance of each species.

N = Total importance value is the sum of the number of individuals observed.

Likewise, the Shapiro-Wilk test was performed to check the normality of data, it indicated that the data were not normally distributed. Therefore, the Mann-Whitney U-test was used to compare the variation of physicochemical parameters of soil and macro-invertebrate abundance between IAPS Invaded and non-invaded habitats.

A Generalized linear Mixed Model (GLMM) (glmer function; poisson family; lme4 package) was used to identify the factors affecting the abundance of macroinvertebrates as the data was highly dispersed to perform a generalized linear model (GLM). Independent variables were soil organic carbon (%), soil organic nitrogen (%), pH, and IAPS Invaded and non-invaded habitat. The dependent variable was an abundance of macroinvertebrates, and the random factor was Area_site. The ‘wiqid’ package (Meredith, 2017) was used for the standardization of variables. All statistical analysis was performed in R program (R Core Team, 2023).

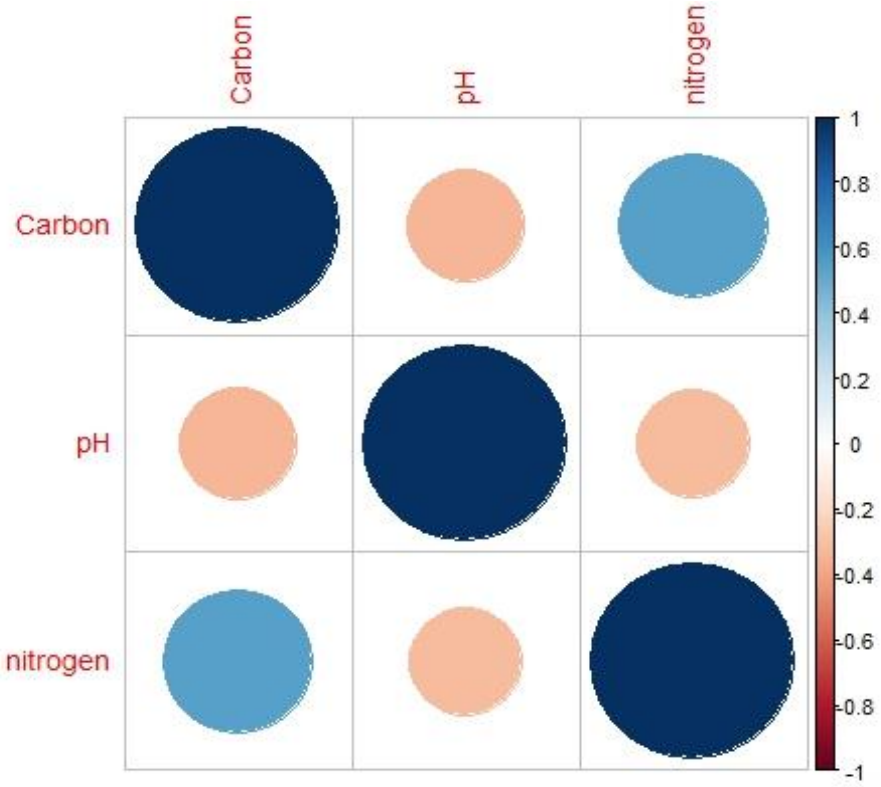

Figure 2: Correlation matrix between predictive variables to estimate factors affecting the diversity of macroinvertebrates in IAPS invaded and non-invaded area of PNP, Nepal

4. Results

A total of 363 specimens of macroinvertebrates were recorded in the six study sites over the sampling period in PNP. These specimens belong to 77 species, 70 genera, and 42 Families under 15 Orders of phyla Annelida (3 specimens) and Arthropoda (360 specimens). However, 69 specimens were of larval stage, and these were excluded from analysis.

4.1 Impact of IAPS on macroinvertebrates

4.1.1 Impact on abundance of macroinvertebrates

In PNP, the result of macroinvertebrates indicates no significant difference (U = 4305.5; p = 0.431) in abundance between invaded and non-invaded habitats. However, higher abundance was recorded for *Pheidole* sp followed by *Odontotermes* sp., *Indotermes* sp., and *Anochetus* sp. in *M. suaveolens* presence habitat. In *M. suaveolens* absence habitat, higher abundance was recorded for *Pheidole* sp., followed by *Meranoplus bicolor* (Table 1).

Table 1: Abundance of macroinvertebrates in invaded and non-invaded habitat

IAPS Invaded habitat	Presence	RA%	Absence	RA%
Mesosphaerum suaveolens	91	38.39	49	20.67
Lantana camara	51	21.51	46	19.40

Likewise, higher abundance in *L. camara* presence habitat was recorded for *Indotermes* sp. Also, higher abundance for *L. camara* absence plots was recorded for *Odontotermes* sp. followed by *Indotermes* sp., *Coptotermes* sp., and *Cormocephalus* sp.

4.1.2 Impact on species richness of macroinvertebrates

In PNP, the results of all macroinvertebrate species indicate no significant difference (W = 4252, p-value = 0.629) in species richness between invaded and non-invaded plots (Figure3). However, number of macroinvertebrate species increased in *M. suaveolens* invaded area while decreased in case of *L. camara* invaded area (Appendix 1).

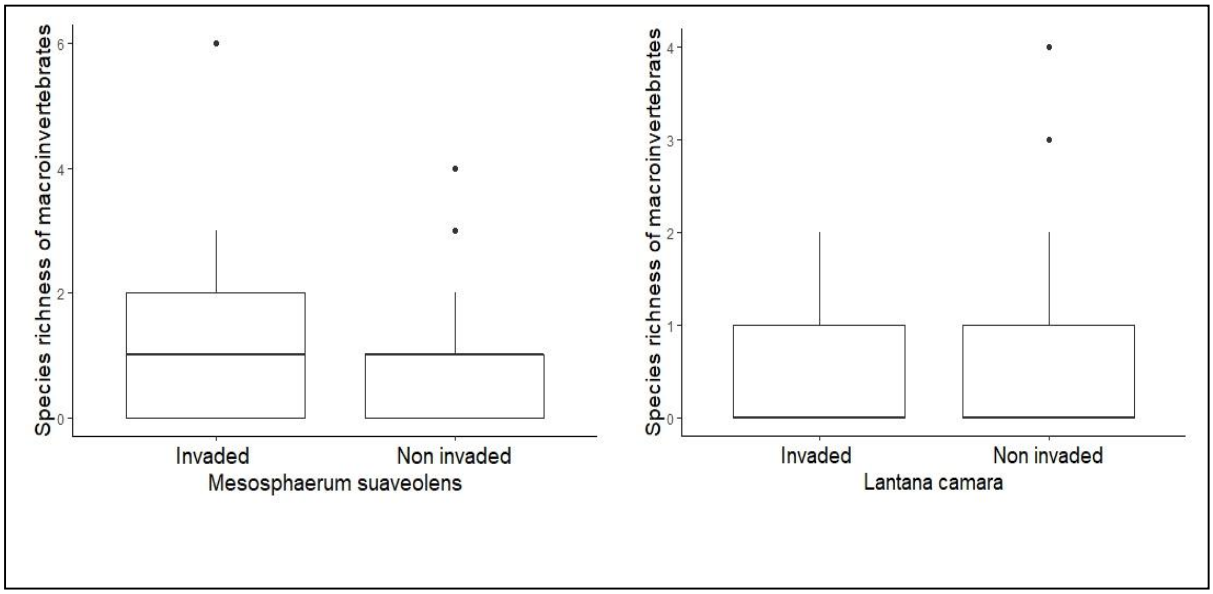

Figure 3: Mean species richness of *Mesosphaerum suaveolens* and *Lantana camara* invaded non-invaded plots in PNP

Macroinvertebrates species like, *Ancyclopus melanocephalus*, *Anochetus* sp., *Anomotarus* sp., *Anthelephila* sp., *Anthicus* sp., Blattidae sp., *Brachyponera chinensis* sp., *Calathus* sp., *Chaetocnema* sp., *Crematogaster* sp., *Delathrum* sp., *Eysarcoris guttigerus, Gonocephalum* sp., *Indotermes* sp., *Lycosidae* sp., *Mecistocephalus* sp., *Melanoxanthus* sp., *Meranoplus bicolor*, *Metochus abbreviatus, Ophonus* sp., Oxyopidae sp., Phalacridae sp., and some species of Archinids like Salticidae sp., Tetragnathidae sp., Thomsidae sp., Zoridae sp. were only present on *M. suaveolens* invaded habitat. While other species like *Aethus* sp., *Almidae* sp., *Apis dorsata*, Endomychidae sp., *Ethmostigmus* sp., *Lithbius* sp., *Metochus abbreviatus* sp., *Monomorium trichomyrmex.*, *Podisus* sp., *Rhadinosa* sp., *Scarites* sp., *Scolopendra* sp., *Sitophilus* sp., *Tetramorium* sp., *Trombidium* sp. were only present on *M. suaveolens* non-invaded habitat. In case of *L.camara* invaded habitat the species richness was found to be lower than non-invaded habitat. Species of Anisolabididae, *Aphaenogaster* sp., *Brachyponera chinensis*, *Camponotus* sp., *Chlaenius* sp., *Dichotomus* sp.1, *Ethmostigmus* sp., *Gonocephalum* sp., *Nylendria* sp., *Scolopendridae* sp. were only found in *L. camara* invaded habitat. Likewise, Acridadae sp., *Anomotarus* sp., Blaberidae sp., *Coccinella septempunctata, crematogster* sp., *Diacamma* sp., *Dysdercus* sp., *Leptogenys leviceps*, Megascolecidae sp., Meranoplus *bicolor*, *Odontoponera denticulata, Odontotermes* sp., *Platynus* sp., *Podisus* sp., *Polyrhacis*

lacteipennis, *Strenosida* sp., *Aulacophora* sp. were only found in non-invaded habitat of *L.camara*.

4.1.3 Impact on diversity of macroinvertebrates

In both IAPS habitats, the average value of Shannon's diversity index was found to be

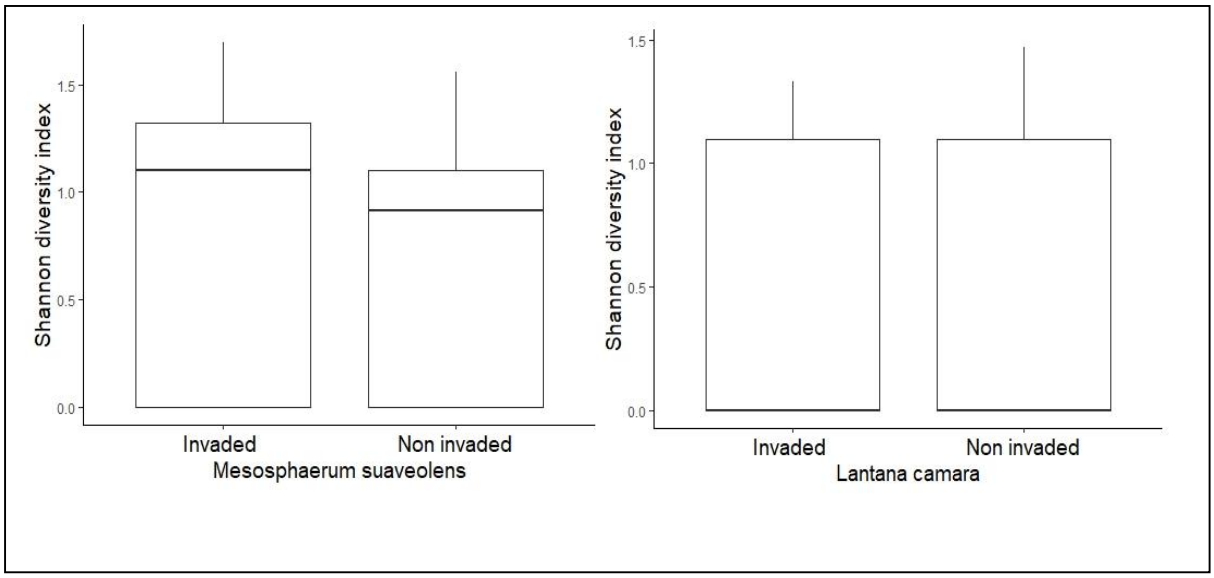

Figure 4: Mean Shannon diversity index for *Mesosphaerum suaveolens* and *Lantana camara* invaded and non-invaded plots in PNP.

higher in invaded area and lower in non-invaded area. The results of Shannon's diversity index indicate no significant difference in species diversity of *M. suaveolens* (W = 1133.5, p = 0.3079) and *L. camara* (W = 895, p = 0.286) invaded and non-invaded plots (Figure 4).

4.2 Impact on soil physico-chemical parameter

Table 2: Comparison of physical parameters in IAPS invaded and non-invaded habitat in PNP

Physical parameters	**Median (Range)**		**Mann Whitney U test**	**p**
	IAPS absence	IAPS presence		
Carbon (%)	1.65(5.637-0.078)	1.007(6.023-0.328)	W = 350	0.115
Nitrogen (%)	0.07(0.196-0.014)	0.08(0.224-0.014)	W = 331	**0.034**
pH	6.13(6.9-5.3)	6.13 (6.8-5.3)	t = 0	1

The soil parameter, nitrogen was varied in the IAPS invaded and non-invaded habitat ($P<0.05$, Table 2). While no variation in the soil organic C and pH ($P> 0.05$) between IAPS invaded and non-invaded habitat.

Table 3: Model-averaged parameters and their Lower Confidence Interval (LCI) and Upper Confidence Interval (UCI) describing factors affecting the abundance of macroinvertebrates. Abundance of macroinvertebrates was the response variable whereas, soil organic carbon (C), pH, soil organic nitrogen (N), and IAPS Invaded and non-invaded were predictive variables. *Significant effects are in bold.

Parameters	**Estimate**	**SE**	**LCI**	**UCI**	**z**	**P**
(Intercept)	-0.072	0.313	-0.640	0.476	-0.231	0.817
C	0.172	0.080	-0.192	0.346	2.137	**0.032**
pH	-0.046	0.075	-0.279	0.248	-0.617	0.537
N	0.046	0.0.074	-0.205	0.288	1.738	**0.082**
IAPSP	0.331	0.133	-0.133	0.459	2.478	**0.013**

The abundance of macroinvertebrates was influenced by C, N, pH, and IAPSP habitat as C, N, and IAPSP had a positive association with abundance of macroinvertebrates and a negative correlation with pH.

5. Discussion

The IAPS such as *M. suaveolens* and *L. camara* alter the soil's physical and chemical properties that influence the diversity and structure of soil invertebrate communities.

5.1 Impact of IAPS on soil macroinvertebrates

The present study identified how IAPS affected the abundance and diversity of macro-invertebrates in PNP. The abundance, richness, and diversity of macroinvertebrates were found higher in case of both *M. suaveolens* and *L. camara* invaded habitat than in non-invaded. The abundance of macroinvertebrates might be influenced by the morphological features and structural complexity of vegetation (Villa Magna, 2009; da Silva & Henry, 2020). However, the effects of invasive plants on the diversity and abundance of soil communities are case-specific (Belnap & Phillips 2001; Kourtev et al., 2003; Callaway et al., 2004).

After introducing IAPS in a healthy ecosystem, they disrupt the fungal mutualistic associations with native plants by changing the nutrient dynamics and interrupting the food webs of soil (Ehrenfeld et al., 2005). As a result, the richness and abundance of soil biota increases due to the invasion of exotic species (Pysek et al., 2012). Vila et al. (2011) synthesized the ecological impacts of plant invasion and found that invasive plants generally reduce the abundance of animal species but have negative effects on their diversity. Similarly, Meisner et al. (2014) showed that invaders favor invertebrates and nematodes.

5.1.1 Impact of *M. suaveolens* on species richness & diversity of macroinvertebrates

In case of *M. suaveolens* invaded habitat the abundance, species richness and diversity of macroinvertebrates were found to be higher than non-invaded habitat. In this study, the *M. suaveolens* had no significant impact on the soil macroinvertebrates. Similarly, soil macroinvertebrates diversity index (Shannon) in invaded areas were diverse than non-invaded ones. It might be due to quick growth and reproduction adaptability of invasive species to different environments, and ability to compete with native flora (Peng et al., 2023). *Mesosphaerum suaveolens* is an aggressive invader reproducing sexually and by perennating roots, which can tolerate harsh environments (Raizada, 2006). Although the allelopathic effect of *Mesosphaerum suaveolens* is not well documented, the presence of essential oils in it may provide a competitive advantage, similar to the allelopathic

properties of Lamiaceae family members such as *Oscimum sanctum*, *Nepata cataria*, and *Salvia species* (Qasem and Foy, 2001). Also, *M. suaveolens* is unpalatable to livestock due to essential oils having 2.3% terpinene 4-ol that make it an undesirable fodder, leading to the loss of other species (Peerzada, 1997).

The variation of abundance and diversity of macro-invertebrates in IAPS invaded area might be due to the litter depth than in non-invaded area. The *M. suaveolens* produces an annual leaf fall of three tons of leaves per hectare (Almeida-Bezerra et al., 2020) and *L. camara* produces more leaf litter (2.15-33.4 ton per hector) that decomposes faster than a litter of native plants, providing more resources to decomposers (Ehrenfeld, 2003; Prescott & Zukswert, 2016). As a result, litter of invasive plants significantly impact specific microbial group's abundance and richness (Zhang et al., 2019). Because the litter provides energy and food sources for the soil community, which determines the complexity and stability of the soil detritus-based food web (Moore et al., 2004). Due to more litter available in the IAPS habitat than on native plants (Ehrenfeld, 2003; Liao et al., 2008), the invasive plant generates more available C and other resources for soil biota than native plants (Prescott & Zukswert, 2016) leading to a more biodiverse soil community (Mayer et al., 2005).

In our study, a species of Millipede (*Delathrum* sp.) which is detritivore, feeding on leaf litter, and also prefer moist habitats such as leaf piles (Wooden et al., 2020) is present only in *M. suaveolens* invaded habitat. *M. suaveolens* consists of a moist habitat with high amount of leaf biomass (Ehrenfeld, 2003).

5.1.2 Impact of *L. camara* on species richness and diversity of macroinvertebrates

In case of *L.camara* there was no significant difference in abundance, species richness and diversity of macroinvertebrates between invaded and non-invaded habitats. However, some species of macroinvertebrates had higher abundance, species richness and diversity in invaded plots. *L. camara* has a greater capacity to trap wind-blown litter because of its dense cover, which helps to build more organic matter content (Everham & Brokaw, 1996). The high abundance of invertebrates at this site could probably be due to leaf litter depth used by some species as a growth substrate for egg-laying and shelter from predators and desiccation (Kazemi et al., 2009; Magura, et al., 2004).

Wiezik et al. (2007) observed an increase in the diversity of ground-dwelling beetles in IAPS invaded habitat due to high habitat complexity through gradual accumulations of

litter and deadwood at various levels of decomposition as well as increased canopy complexity leading to deep litter layers. This study supports our results, as species of ground-dwelling beetles of family like Carabidae, Chrysomelidae, Scarabaeidae, Tenebrionidae, Staphylinidae, Phalacridae, and Anthicidae had higher species richness and diversity in IAPS invaded habitat. Furthermore, these litter layers offer increased resources and structural complexity, microclimate diversity, refugia for protection from predators, increasing prey availability and also provide a buffering effect against temperature fluctuations (Magura, et al., 2004; Wiezik et al., 2007; Kazemi et al., 2009).

In IAPSA habitat of this study, it was a grazing area for domestic livestock. Grazing has a severe effect on native vegetation leaving habitats with little ground cover (Hobbs, 1987) which in turn affects the soil and litter layer causing changes in structure and composition of invertebrate communities (Majer, 1983). Removal of the ground vegetation and litter represents a reduction in the quantity of food and habitat (Harvey & Yen, 1989) which may directly affect abundance of the taxa that rely on those resources such as millipedes. So, grazing activities cause a decline in the abundance of litter and topsoil invertebrates (Abbott, 1989; Majer, 1989).

In this study, species of termites like *Coptotermes* have greater ecological effects were found only in IAPS non-invaded plots. They have a larger colony population and hollow-out live trees (Evans, 2021). There is little research completed on the long-term effects of these invasive termites on natural ecosystems. However, it is possible to make predictions based on the known biology and differences in their life history characters. Likewise, Chilopods have relatively higher tolerance and diversity in adaptation (Yin et al., 2018; 2019) so they were present in both IAPS invaded and non-invaded habitats. However, grazed areas may have a less diverse ground invertebrate fauna because they do not provide the same breadth of resources or as many possible niches as those with woodland. Therefore, this might be the reason for low abundance of macroinvertebrates in non-invaded habitat than in invaded of PNP. However, a study has found that twigs and leaves browsed by mammalian herbivores increase herbivorous insects on twigs; due to changes in the plant tissue's chemistry, morphology, and growth rate (Cheli et al., 2010). So, this might be the reason for the existing abundance and richness of macroinvertebrates in IAPS non-invaded plots

5.2 Impact on physico-chemical parameters of soil

The findings of this study showed signs of soil quality degradation in the IAPS-invaded habitat of PNP. The analysis of physicochemical parameters confirmed that, except for pH, C and N varied significantly in the IAPS-invaded and non-invaded habitats.

In this study, the abundance of macroinvertebrates was influenced by C and N soil content and had a positive association within IAPS invaded habitat. The increase in the concentration of C and N in this study might result from a high amount of leaf biomass under a canopy of *M. suaveolens* and *L. camara* (Simba et al., 2013). The concentration of litter C and N of invasive plants is often higher than that of native plants (Liao et al., 2008). Invasive plants can affect soil chemical components by increasing biomass or releasing allelochemicals (Kourtev et al., 1998). Mandal & Guantum, (2014) state that the increase in C and N concentration with an increase in *L. camara* could be due to a decrease in nutrient-impounding followed by a reduction of native species of that region. Afreen et al., (2018) demonstrated similar results in which invaded plots had more available total N. It was likely that *M. suaveolens* patches did not absorb as much nutrients as patches with more diverse species. Still, diverse native species may require more nutrients, resulting in lower concentration soil (Mapaura et al., 2024). Similarly, Sharma (2011) found an increase in concentration of C and N in *L. camara*-invaded region. IAPS promotes accumulation of litter under the shrubs resulting in a build-up of organic C and N (Sharma & Raghubanshi, 2009). Therefore, greater C and N stocks have been observed in the invaded than in native ecosystems (Vitousek & Walker, 1989; Hibbard et al., 2001). The increase in N concentration with an increase in litter below the canopy of IAPS directly favors a suitable microclimate (Wekhanya et al., 2020; Ehrenfeld, 2003; Prescott & Zukswert, 2016). This leads to an increase in abundance of macroinvertebrates (Mayer et al., 2005; Pysek et al., 2012; Meisner et al., 2014).

Similarly, the abundance of macroinvertebrates was negatively associated with pH of soil in IAPS invaded area of PNP. Salmon et al. (2008) showed that lower the pH value, lesser the faunal abundance. On the contrary, Wang et al. (2015) showed that the abundance of soil invertebrates is negatively associated with pH of soil. A study by Yin et al. (2018) also supports our result that an increase in pH of soil limits the growth and reproduction of soil fauna. As species of soil fauna have different preferences, their association with soil pH might differ (Kautz et al., 2006). Also, fluctuations in the nutrient availability and

adsorption quality of soil driven by pH indirectly affect the microclimate of soil fauna (Salmon et al., 2008; Mulder & Elser, 2009). However, pH of soil primarily depends on the identity of the invasive species (Timsina et al., 2011; Darji et al., 2021). IAPS makes soil acidic which is less nutrient thereby providing less suitable environments for invertebrates (Kazemi, et al., 2009).

As the soil communities are affected by biotic and abiotic components like litter quality and other physico-chemical parameters of the soil, the abundance of the fauna is dependent upon such components (Frouz et al., 2008; Korboulewsky et al., 2016). One of the major reasons for this can be the soil compaction caused by roots of trees, trampling and forest management practices, and presence of sandy loam soil. Certain taxa such as Dipluran, Coleopteran adults, Chilopoda, Diplopoda, and Diptera larvae negatively react to soil compaction (Menta, 2012). This might be the reason for the lower abundance of these taxa in IAPSA area due to soil compaction by grazing activities. In this study earthworm species were only present in IAPS non-invaded habitats because they favor a less acidic and arid environment (Phillips et al., 2019). According to our sampling design, we dig soil up to 10 cm dig. So, this might be the reason for the presence of earthworm species of family Megascolecidae, Lumbricidae, and Almidae that live on upper soil layer (Nguyen et al., 2020; Blakemore et al., 2009). However, the earthworm of family Almidae was observed in sampling area which was near the riverbank in Jirabhawani Rural Municipality Ward No. 1 Shanti tole. As this family prefers semiaquatic terrestrial habitat (Chanabun et al., 2020).

In case of soil arthropods, their communities were affected depending on the invasive plant or on which microclimate of the plant was sampled such as *Microstegium vimineum* invasion reduces tick survival due to high temperature and low humidity in invaded habitat (Effah et al., 2020; Simao et al., 2010). Some species in this study occurred across all sites and therefore have good potential for use as bioindicators for examples; Hymenoptera (*Camponotus* sp, *Pheidole* sp, *Technomyrmex* sp), Blattodea (*Indotermes* sp., *Odontotermes* sp., *Coptotermes* sp).

5.3 Implications for Management

Biological invasion is positively correlated with human population density, which means where the population density is high there is a high chance of spreading of IAPS (Spear et al., 2013). IAPS populations are also more common in areas with a high population density,

roadsides, open grasslands, open forests and fallow lands. Plant invasion impacted forest edges, roadside vegetation, watercourse, and riverine forests (Sharma et al., 2017; Afreen et al., 2018; Bhatta et al., 2020). The most effective management strategy for preventing plant invasion in PAs is to target the most harmful species, detect them early, and monitor them on a regular basis. The obtained results from this study demonstrate that *M. suaveolens* and *L. camara* had a negative impact on species richness and diversity as well as soil characteristics. These IAPS significantly decreases species richness and species diversity of invaded plots by changing soil characteristics suggesting that this invasive species should be controlled strictly in PNP. From this study, park managers should be aware of these species for early detection and eradication from the core area of PA. As a result, IAPS require continuous monitoring and management. The present study has revealed that *M. suaveolens* and *L. camara* was spreading rapidly in grassland of PNP. Due to the unpalatable nature of *M. suaveolens* and *L. camara* the population remains massively high in invaded sites. As a result, eradicating these IAPS population not only safeguards the protected area from its harmful effects, but also prevents biodiversity loss, grazing area loss, and socioeconomic consequences. Controlled fire and entire uprooting of this species just before flowering are required for successful management. Similarly, regular field monitoring is critical for early detection of this species in small patches in a new location and rapid removal. Among manual or mechanical, chemical, and biological methods, mechanical methods, i.e., uprooting the whole plant before flowering, can be good methods for the management of *M. suaveolens and L. camara* (Sharma et al., 2005).

6. Conclusions and recommendations

6.1 Conclusions

The present study helped in concluding that soil macroinvertebrate abundance, species richness, and diversity were influenced by presence of IAPS and change in physico-chemical parameters of soil. Also, PNP have been affected by *M. suaveolens* and *L. camara* invasion; including the core area of the park. They can grow easily in dry soil habitat of roadside, abandoned, fallow lands, and open grasslands and cause changes in above ground vegetation and belowground soil characteristics. The variance of macro-invertebrate abundance in PNP between the IAPS invaded and non-invaded habitats is predominantly caused by alterations of the structural composition and quality of soil. The change in C, N, and pH concentration indicates the possibility of variation in macro-invertebrate diversity between two habitats of PNP. The occurrence of some species of macroinvertebrates was supported by *M. suaveolens* and *L. camara* while the negative effect was noticed on other species of the order Ophisthopora (Almidae sp, Endomychidae sp, and Megascolecidae sp). Overall, this study concludes that the *M. suaveolens* and *L. camara* invasion significantly reduces the species richness and species diversity, which alters the species composition of macroinvertebrates and causes changes in soil characteristics.

6.2 Recommendations

- Species-level identification of the soil macroinvertebrates should be done.
- In present study, higher diversity in the invaded sites may suggest that *M. suaveolens* & *L. camara* invasion has a positive role in diversity of macroinvertebrates
- So, there needs to beinvestigate the specific mechanism to identify if invasive species provide better habitat and food resources or had just altered soil chemistry in a way that benefits certain macroinvertebrates.
- More soil variables, such as soil microbial analysis and soil physical analysis such as soil texture, bulk density, soil temperature, and soil chemical analysis such as potassium, phosphorus, and soil moisture, should be investigated in different season to fully analyze the impact of *M. suaveolens* and *L. camara* on soil.

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Appendices

Appendix 1: Abundance of macroinvertebrates in IAPS Invaded and non-invaded habitats of PNP, Nepal**.**

S. N	Phylum	Order	Family	Scientific name	Mean±SD (IAPSP)	Mean±SD (IAPSA)
1.	Arthropoda	Hymenoptera	Formicidae	*Anochetus sp.*	5±0.00	
2.	Arthropoda	Hymenoptera	Formicidae	*Aphaenogaster sp.*	1±0.00	
3.	Arthropoda	Hymenoptera	Formicidae	*Brachyponera chinensis*	1.5±0.5	
4.	Arthropoda	Hymenoptera	Formicidae	*Brachyponera sp.1*	1±0.00	
5.	Arthropoda	Hymenoptera	Formicidae	*Camponotus opaciventris*	1±0.00	1±0.00
6.	Arthropoda	Hymenoptera	Formicidae	*Camponotus tanaemyrmex*	3±0.00	
7.	Arthropoda	Hymenoptera	Formicidae	*Camponotus sp.1*	1.33±0.47	1±0.00
8.	Arthropoda	Hymenoptera	Formicidae	*Carebara sp.*	1±0.00	1±0.00
9.	Arthropoda	Hymenoptera	Formicidae	*Crematogaster sp.*	1±0.00	1±0.00
10.	Arthropoda	Hymenoptera	Formicidae	*Nylanderia sp.*	2±0.00	
11.	Arthropoda	Hymenoptera	Formicidae	*Pheidole sp.*	2.14±0.64	1.44±1.25
12.	Arthropoda	Hymenoptera	Formicidae	*Diacamma sp.*		1±0.00
13.	Arthropoda	Hymenoptera	Formicidae	*Leptogenys leviceps*		1±0.00
14.	Arthropoda	Hymenoptera	Formicidae	*Leptogenys sp.1*		1±0.00
15.	Arthropoda	Hymenoptera	Formicidae	*Meranoplus bicolor*		3±1.63
16.	Arthropoda	Hymenoptera	Formicidae	*Monomorium trichomyrmex*		1±0.00
17.	Arthropoda	Hymenoptera	Formicidae	*Odontoponera denticulata*		2±0.00

18.	Arthropoda	Hymenoptera	Formicidae	*Polyrhacis lacteipennis*		1±0.00
19.	Arthropoda	Hymenoptera	Formicidae	*Tetramorium sp.*		1±0.00
20.	Arthropoda	Hymenoptera	Apidae	*Apis dorsata*	2±0.00	
21.	Arthropoda	Coleoptera	Endomychidae	*Ancyclopus melanocephalus*	1±0.00	
22.	Arthropoda	Coleoptera	Carabidae	*Anomotarus sp.*	1±0.00	1±0.00
23.	Arthropoda	Coleoptera	Carabidae	*Calathus sp.*	1±0.00	
24.	Arthropoda	Coleoptera	Carabidae	*Chlaenius sp.*		
25.	Arthropoda	Coleoptera	Carabidae	*Ophonus sp.*	1±0.00	
26.	Arthropoda	Coleoptera	Carabidae	*Platymetophus sp.*	2±0.00	
27.	Arthropoda	Coleoptera	Carabidae	*Platynus sp.*	1±0.00	1±0.00
28.	Arthropoda	Coleoptera	Carabidae	*Scarites sp.*		1±0.00
29.	Arthropoda	Coleoptera	Anthicidae	*Antheliphila sp.*	1±0.00	
30.	Arthropoda	Coleoptera	Chrysomelidae	*Anthicus sp.*	1±0.00	
31.	Arthropoda	Coleoptera	Chrysomelidae	*Aulacophora sp.*	1± 0.00	
32.	Arthropoda	Coleoptera	Chrysomelidae	*Chaetocnema sp.*	1±0.00	
33.	Arthropoda	Coleoptera	Chrysomelidae	*Rhadinosa sp.*		1±0.00
34.	Arthropoda	Coleoptera	Scarabaeidae	*Dichotomius sp.*	1±0.00	
35.	Arthropoda	Coleoptera	Tenebrionidae	*Gonocephalum sp.*	1±0.00	
36.	Arthropoda	Coleoptera	Tenebrionidae	*Strenosida sp.*		1±0.00
37.	Arthropoda	Coleoptera	Staphylinidae	*Philonthus sp.*	1±0.00	
38.	Arthropoda	Coleoptera	Phalacridae	*Phalacridae*	1±0.00	
39.	Arthropoda	Coleoptera	Coccinellidae	*Coccinella septempunctata*		1±0.00
40.	Arthropoda	Coleoptera	Elateridae	*Melanoxanthus sp.*		1±0.00
41.	Arthropoda	Coleoptera	Curcullionidae	*Sitophilus sp.*		1±0.00

42.	Arthropoda	Hemiptera	Pentatomidae	*Eysarcoris guttigerus*	1±0.00	
43.	Arthropoda	Hemiptera	Pentatomidae	*Podisus sp.*		1±0.00
45.	Arthropoda	Hemiptera	Cydnidae	*Aethus sp.*		1±0.00
46.	Arthropoda	Hemiptera	Pyrrhocoridae	*Dysdercus cingulatus*		1±0.00
47.	Arthropoda	Hemiptera	Rhyparochromidae	*Metochus abbreviatus*		1±0.00
48.	Arthropoda	Orthoptera	Acridadae	*Acridadae sp.*	1±0.00	1±0.00
49.	Arthropoda	Orthoptera	Gryllidae	*Gryllidae sp.*	1±0.00	1±0.00
50.	Arthropoda	Blattodea	Indotermitidae	*Indotermes sp.*	8±2.63	4±2.00
51.	Arthropoda	Blattodea	Termitidae	*Odontotermes*	2.8±5.89	2.4±1.01
52.	Arthropoda	Blattodea	Rhinotermitidae	*Coptotermes sp.*		2±1.00
53.	Arthropoda	Blattodea	Blattidae	*Blattidae sp.*	1±0.00	
54.	Arthropoda	Dermaptera	Anisolabididae	*Anisolabididae sp.*	1±0.00	
55.	Arthropoda	Arachnida	Oxyopidae	*Oxyopidae sp.1*	1±0.00	2±0.00
56.	Arthropoda	Araneae	Oxyopidae	*Oxyopidae sp.2*	1±0.00	1±0.00
57.	Arthropoda	Araneae	Oxyopidae	*Oxyopidae sp.3*	1±0.00	1±0.00
58.	Arthropoda	Araneae	Thomsidae	*Thomsidae sp.1*	1±0.00	
59.	Arthropoda	Araneae	Thomsidae	*Thomsidae sp.2*	1±0.00	
60.	Arthropoda	Araneae	Tetragnathidae	*Tetragnathidae sp.1*	1±0.00	
61.	Arthropoda	Araneae	Pholcidae	*Pholcus sp.*	1±0.00	
62.	Arthropoda	Araneae	Lycosidae	*Lycosidae sp.1*	1±0.00	2±0.00
63.	Arthropoda	Araneae	Zodaridae	*Zodaridae sp.1*	1±0.00	
64.	Arthropoda	Araneae	Salticidae	*Salticidae sp.1*	1±0.00	
65.	Arthropoda	Araneae	Lycosidae	*Agelenidae sp.1*	1±0.00	1±0.00
66.	Arthropoda	Araneae	Scytodidae	*Scytodidaes sp. 1*	1±0.00	1±0.00

67.	Arthropoda	Geophilomorpha	Mecistocephalidae	*Mecistocephalus sp.*	1±0.00	
68.	Arthropoda	Lithobiomorpha	Lithobiidae	*Lithobius sp.*	1±0.00	1±0.00
69.	Arthropoda	Scolopendromorpha	Scolopendridae	*Scolopendra sp.*	1±0.00	1±0.00
70.	Arthropoda	Scolopendromorpha	Scolopendridae	*Cormocephalus sp.*	1±0.00	1±0.00
71.	Arthropoda	Scolopendromorpha	Scolopendridae	*Ethmostigmus sp.*	1±0.00	1±0.00
72.	Arthropoda	Polidesmida	Paradoxosomatidae	*Delathrum sp.*	1±0.00	
73.	Arthropoda	Acariformes	Trombidiidae	*Trombidium sp.*	1±0.00	1±0.00
74.	Arthropoda	Isopoda	Oniscidea	*Oniscidea sp.*	1±0.00	1±0.00
75.	Annelida	Ophisthopora	Almidae	*Almidae sp.*		1±0.00
76.	Annelida	Ophisthopora	Lumbricidae	*Lumbricidae sp.*		1±0.00
77.	Annelida	Ophisthopora	Megascolecidae	*Megascolecidae sp.*		1±0.00

Appendix 2: Model-averaged parameters and their Lower Confidence Interval (LCI) and Upper Confidence Interval (UCI) confidence limits describing factors affecting the abundance of macroinvertebrates in IAPSP habitat. Abundance of macroinvertebrates was the response variable whereas C, N, and pH were predictive variables.

*Significant effects are in bold.

Habitat	**Parameters**	**Estimate**	**SE**	**LCI**	**UCI**	**z**	**p**
IAPSP	Intercept	0.034	0.376	-0.702	0.772	0.303	0.926
	C	0.399	0.115	0.172	0.626	1.338	**0.0005**
	pH	-0.063	0.103	-0.266	0.140	-1.975	0.542
	N	0.194	0.099	-0.0003	0.388	2.132	**0.050**

Appendix 3: Model-averaged parameters and their Lower Confidence Interval (LCI) and Upper Confidence Interval (UCI) confidence limits describing factors affecting the abundance of macroinvertebrates in IAPSA habitat. Abundance of macroinvertebrates was the response variable whereas C, N, and pH were predictive variables.

*Significant effects are in bold.

Habitat	**Parameters**	**Estimate**	**SE**	**LCI**	**UCI**	**z**	**p**
IAPSA	Intercept	0.030	0.350	-0.534	0.528	0.087	0.930
	C	0.049	0.132	-0.219	0.398	0.372	0.710
	pH	0.047	0.132	-0.206	0.352	0.356	0.722
	N	-0.021	0.123	-0.219	0.309	-0.173	0.863

Appendix 4: List of species of macroinvertebrates deposited in the CDZMTU, Kirtipur Nepal

Taxa	**No. of specimen**	**Plot no.**	**Date of collection**
Haplotaxida			
Almidae sp.	1	41A	2023/11/05
Lumbricidae sp.	1	43A	2023/11/05
Megascolecidae sp.	1	78A	2023/11/10
Acari			
Trombidium sp.	2	20A, 30P	2023/11/04
Chilopoda			
Mecistocephalus sp.	1	8P	2023/11/03
Scolopendra sp.	3	58P, 2A, 39A	2023/11/03-2023/11/09
Lithobius sp.	2	2A, 78P	2023/11/02-2023/11/11
Cormocephalus sp.	5	48A, 74A, ,82P, 86P	2023/11/08-2023/11/11
Ethmostigmus sp.	2	89P, 30A	2023/11/04-2023/11/11
Diplopoda			
Delathrum sp.	1	21P	2023/11/04
Crustacea			
Oniscidea sp.	2	7A, 32P	2023/11/04-2023/11/05

Coleoptera			
Ancyclopus sp.	1	30P	2023/11/04
Anomotarus sp.	2	22P, 59A	2023/11/04-2023/11/09
Anthelephila sp.	1	36P	2023/11/05
Anthicus sp.	1	23P	2023/11/04
Aulacophora sp.	1	30P	2023/11/04
Calathus sp.	1	10P	2023/11/03
Chaetocnema sp.	1	5P	2023/11/03
Chlaenius sp.	1	51P	2023/11/08
Coccinella septempunctata	1	65A	2023/11/10
Dichotomius sp.	1	79P	2023/11/11
Gonocephalum sp.	4	6P, 14P, 46P, 80P	2023/11/03-2023/11/11
Melanoxanthus sp.	1	23A	2023/11/04
Ophonus sp.	1	17P	2023/11/04
Phalacridae sp.	1	42P	2023/11/05
Platynus sp.	2	14A, 16P	2023/11/03-2023/11/04
Philonthus sp.	4	5P, 7P, 17P, 29P	2023/11/03-2023/11/04
Platymetopus sp.	2	18P	2023/11/03
Rhadinosa sp.	1	8A	2023/11/03
Scarites sp.	1	10A	2023/11/03

Sitophilus sp.	1	1A	2023/11/02
Strenosida sp.	1	72A	2023/11/10
Orthoptera			
Acridadae sp.	5	13P, 16P, 22A, 32A, 49A	2023/11/03-2023/11/08
Gryllidae sp.	4	24P, 73P, 37A, 77A	2023/11/03-2023/11/11
Hemiptera			
Eysarcoris guttigerus	1	30P	2023/11/04
Metochus abbreviatus	1	2A	2023/11/02
Podisus sp.	1	19A	2023/11/04
Aethus sp.	1	24A	2023/11/04
Dysdercus sp.	1	48A	2023/11/08
Blattodea			
Coptotermes sp.	4	50A, 51A	2023/11/08
Indotermes sp.	48	2P, 15P, 32P, 46P, 49P, 48A, 49A	2023/11/02-2023/08
Odontotermes sp.	26	10P, 11P, 27P, 39P, 43P, 1A, 51A, 52A, 56A, 60A	2023/11/03
Blattidae sp.	1	28P	2023/11/04
Dermaptera			
Anisolabididae sp.	1	79P	2023/11/11
Hymenoptera			
Anochetus sp.	5	37P	2023/11/05

Apis dorsta	2	45A	2023/11/05
Aphaenogaster sp.	1	50P	2023/11/08
Brachyponera chinensis	3	17P, 88P	2023/11/04-2023/11/11
Brachyponera sp.1	1	89P	2023/11/11
Camponotus opaciventris	2	18P, 16A	2023/11/04
Camponotus sp.1	5	41P, 55P, 58P, 45A	2023/11/05-2023/11/09
Camponotus tanaemyrmex	3	36P	2023/11/05
Carebara sp.	3	90P, 46A	2023/11/08-11/11
Crematogaster sp.	3	7P, 42P, 78A	2023/11/03-2023/11/11
Diacamma sp.	1	53A	2023/11/09
Leptogenys leviceps	1	75A	2023/11/10
Leptogenys sp.1	1	82A	2023/11/11
Meranoplus bicolor	5	7A, 49A	2023/11/03-2023/08
Monomorium trichomyrmex	1	36A	2023/11/05
Nylanderis sp.	2	88P	2023/11/11
Odontoponera denticulate	2	81A	2023/11/11
Pheidole sp.	28	11P, 28P, 31P, 32P, 36P, 56P, 2A, 3A, 14A, 18A, 19A, 38A, 39A, 71A	2023/11/02-2023/11/09
Polyrhacis lacteipennis	1	66A	2023/11/10

Tetramorium sp.	1	19A	2023/11/04
Aranea			
Agelenidae sp.1	1	48A	2023/11/08
Lycosidae sp.1	3	37P, 23A	2023/11/04-2023/11/05
Oxyopidae sp.1	3	5P, 1A	2023/11/02-2023/11/03
Oxyopidae sp.2	2	7P, 8A	2023/11/02
Oxyopidae sp.3	2	13P, 20A	2023/11/03-2023/11/04
Pholcus sp.	1	30P	2023/11/04
Scytodidae sp.1	1	32A	2023/11/05
Salticidae sp.1	1	42P	2023/11/05
Thomsidae sp.1	1	8P	2023/11/03
Tetragnathidae sp.1	2	23P	2023/11/04
Thomsidae sp.2	1	30P	2023/11/04
Zoridae sp.1	1	39P	2023/11/05

Appendix 5: Photographs of identified macroinvertebrate specimens within study plots

1. *Scarites sp.* (CDZMTU-GEN- 111)

2. *Platynus* sp.

3. Anomotarus sp.

4. Chlaenius sp.

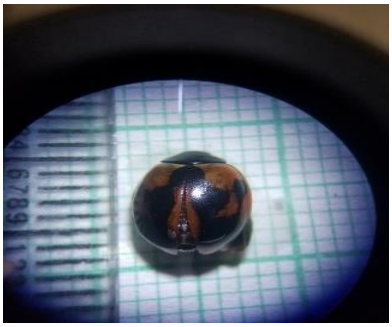
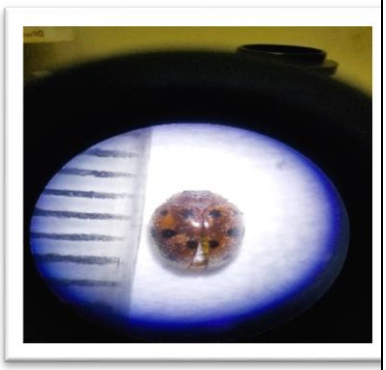
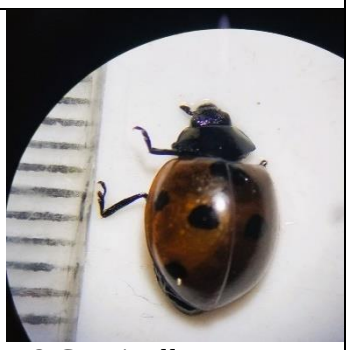
5. Ophonus sp.

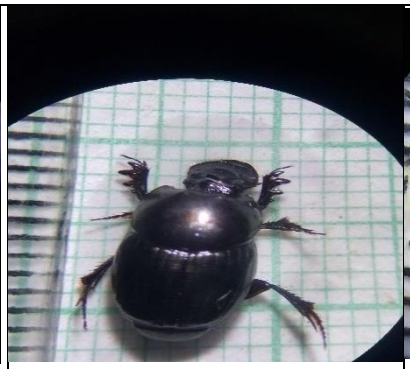
6.Platymetopus sp. (CDZMTU-GEN-107)

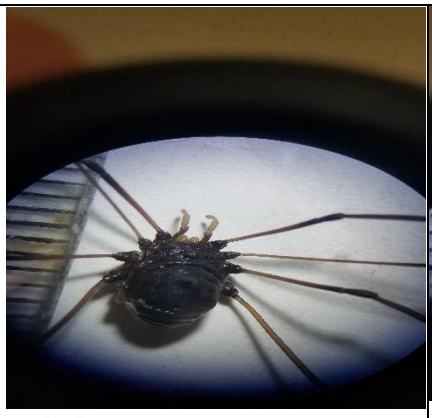
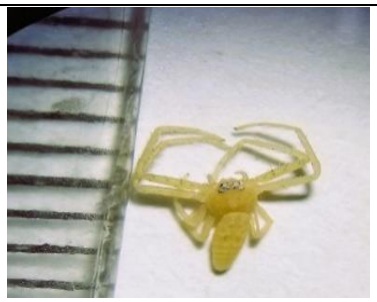
7. Calathus sp.

8. Gonocephalum sp. (CDZMTU-GEN-108)

9. Strenosida sp.

 *10. Anthelephila sp.*	 *11. Chaetocnema sp.*	 *12. Rhadinosa sp.*
 13. *Aulacophora* sp. (CDZMTU-GEN-106)	 *14. Chrysomelidae sp.*	 *15. Sitophilus sp.*
 *16.Coccinella trasversalis*	 *17.Coccinella septempunctata*	 *18.Coccinella septempunctata*

		
19. Ancylopus sp. (CDZMTU-GEN-105)	*20.Dichotomius sp.*	*21. Philonthus* sp. (CDZMTU-GEN-109)
		
22. Melanoxanthus sp. (CDZMTU-GEN-110)	*23. Phalacridae sp.*	24.Eysarcoris guttigerus (CDZMTU-HET-201)
		
25. Courtesius sp.	*26. Metocus abbreviatus* (CDZMTU-HET-202)	*27.*Lycosidae (CDZMTU-GEN-119)

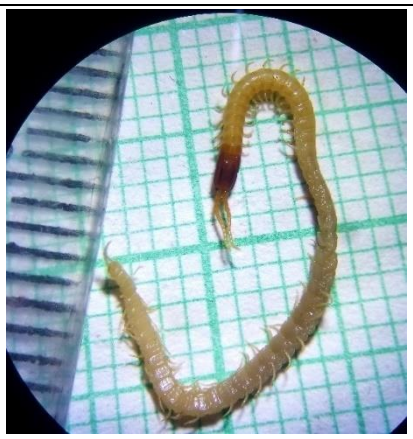
 28.Pholcus sp. (CDZMTU-GEN-121)	 29. Salticidae	 30.Thomsidae (CDZMTU-GEN-122)
 31.Oxyopidae (CDZMTU-GEN-120	 32. Zodarridae	 33. Scytodidae
 *34. Tetragnathidiae sp*	 35. Aglenidae	 36.Gryllidae (CDZMTU-GEN-112)

37. Acridadae

38. Blattdae

39. Scolopendra sp.

(CDZMTU-GEN-102)



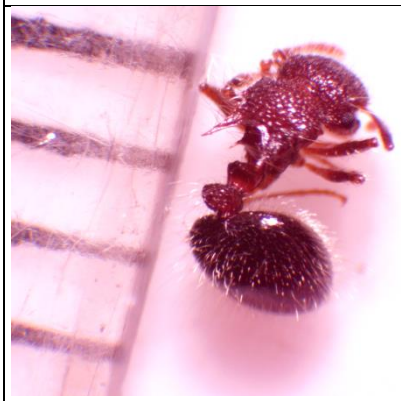
40.*Mecistocephalus* sp.

(CDZMTU-GEN-101)

41. *Ethmostigmus* sp.

42. *Delathrum* sp.

(CDZMTU-GEN-104)



43. *Pheidole major*

44. *Pheidole minor*

(CDZMTU-GEN-118)

45.Polyrhacis lacteipennis

46. Meranoplus bicolor

47. Crematogaster sp.1

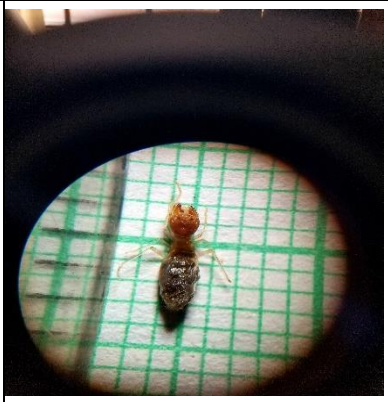
49. *Camponotus* sp. (CDZMTU-GEN-117)

48. Camponotus opaciventris

50. *Brachyponera chinensis* (CDZMTU-HYM-501)

51. *Odontotermes* sp (CDZMTU-GEN-116)

52. *Coptotermes* sp (CDZMTU-GEN-114)



53. *Indotermes* sp (CDZMTU-GEN-115)

Appendix 6: Photographs during field and lab work

1. Sieving of macroinvertebrates on *L. camara* invaded site

2. Identification of macroinvertebrates on the laboratory

3. Vials used for sample collection

4. M. suaveolens invaded site

Appendix 7: Approval letter from Parsa National Park

नेपाल सरकार
वन तथा वातावरण मन्त्रालय
राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभाग
पर्सा राष्ट्रिय निकुञ्ज कार्यालय
आद्याभार, बारा

पत्र संख्या: २०८०/०८१
चलानी नं.: ४४६

मिति : २०८०/०७/१४

विषय : अध्ययन अनुसन्धान अनुमति सम्बन्धमा।

श्री भरत बाबु श्रेष्ठ ,
सहिद लखन गाउँपालिका-९, गोरखा ।

प्रस्तुत विषयमा राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण विभागको पत्र संख्या २०७९/०८० चलानी नं. २१५२ मिति २०७९/१०/०३ गतेको पत्रानुसार यस पर्सा राष्ट्रिय निकुञ्जमा निम्नानुसारको अध्ययन अनुसन्धान अनुमति दिईएको व्यहोरा अनुरोध गर्दछु।

अनुसन्धानकर्ताको नाम	Bharat Babu Shrestha		
ठेगाना	सहिद लखन गाउँपालिका-९, गोरखा	ईमेल:shresthabb@gmail.com	मो. नं. ९८४१२४१४८४
सम्बद्ध संस्था	Central Department of Botany, Tribhuvan University		
अनुसन्धानको प्रकृति	संस्थागत		
पद	Professor		
अनुसन्धानको शिर्षक	Threat Analysis and Management options of Invasive Alien Species in Protected Areas of Nepal		
अनुसन्धान विधि	Biodiversity data compilation, ecological Modeling , social survey and consultation, camera traps, species distribution mapping and ecological impact studies	नमुना संकलन गर्ने	नमुना परिक्षण गर्ने स्थान नेपाल
अनुसन्धानको अवधि	२०८०/०७/१५ गते देखि २०८०/०९/२९ गते सम्म		

शर्तहरु :

१. अनुसन्धानकर्ताले राष्ट्रिय निकुञ्ज तथा वन्यजन्तु संरक्षण ऐन २०२९ र नियमावली २०३० तथा मातहतका सवै नियमावलीहरुको पूर्ण पालना गर्नु पर्नेछ।
२. अध्ययन अनुसन्धान गर्दा यस कार्यालयको कर्मचारीको प्रत्यक्ष रोहवरमा गर्नु पर्नेछ।
३. अनुसन्धानकर्ताले आफ्नो अनुसन्धानको प्रस्ताव यस कार्यालयमा पेश गर्नु पर्नेछ।
४. अनुसन्धानकर्ताले अनुसन्धान समाप्त भएपछि एक प्रति प्रतिवेदन र एक प्रति ईलोक्ट्रोनिक प्रतिवेदन यस कार्यालयमा अनिवार्य रुपमा उपलब्ध गराउन पर्नेछ।
५. यस कार्यालयले तोकेको ठाउँमा मात्र नमुना संकलन गर्नु पर्नेछ र संकलित नमुना विदेश लैजान पाईने छैन्।
६. नमूना संकलन गर्दा हर्वेरियम प्रयोगशाला अभिलेखिकरण तथा ल्याब परिक्षण प्रयोजनका लागि जम्मा २ वटा मात्र नमूना संकलन गर्नु पर्नेछ।
७. तोकिएको शर्तहरुको पालना नगरेमा विभागले कुनै पनि समयमा यो अनुमति रद्द हुनेछ।
८. बाँकीको हकमा प्रचलित कानुन बमोजिम हुनेछ।
९. गाडी नं. बा १६ प २२४३ (स्कर्पियो)

२०८०/७/१४
सन्तोष कुमार भगत
निमित्त वरिष्ठ संरक्षण अधिकृत
नि. वरिष्ठ संरक्षण अधिकृत

बोधार्थ :
श्री सबुज गण गण, आधाभार ब्यारेक : जानकारीका लागि अनुरोध छ।
सिनियर गेमस्काउट श्री सन्तोष कुमार यादव : अनुसन्धान कार्यको रोहवरमा रही प्रतिवेदन पेश गर्न हुन ।

फोन नं.-522051-053 , ०५३-522046
ईमेल- info@parsanationalpark.gov.np

Appendix 8: Codes run in R-programming

```
setwd("C:/Users/Hp/Desktop/Hari sir send files")

list.files()

####glmm for presence data

cp=read.csv("IAPSP plot all data(AutoRecovered).csv", header = T, sep = ",")

Cp

####install.packages(AER)

library(AER)

####install.packages("wiqid")

library(wiqid)

library(lme4)

glmm_presence<-
glmer(Abundance~standardize(Carbon)+standardize(pH)+standardize(Nitrogen)+(1|Ar
ea_site),data=cp,family="poisson")


??glmm

summary(glmm_presence)

Confint(glm_presence)


###glmm for absence data

Ap=read.csv("IAPSA plot all data.csv", header = T, sep = ",")

Ap

library(AER)

library(lme4)

glmm_Absence<-glmer(Abundance~
standardize(Carbon)+standardize(pH)+standardize(Nitrogen)+
(1|Area_site),data=Ap,family="poisson")


??glmm

summary(glmm_Absence)

confint(glm_Absence)


####glmmfor both data
```

```
df=read.csv("All data of thesis.csv", header = T, sep = ",")

Df

library(AER)

library(lme4)

glmm_both<-glmer(Abundance~
standardize(Carbon)+standardize(pH)+standardize(nitrogen)+Invasive.species+(1|Area
_site),data=df,family="poisson")


??glmm

summary(glmm_both)

confint(glm_both)


###3normality test

cp=read.csv("Invasive absence presence.csv", header = T, sep ="," )

head(cp)

shapiro.test(cp$Carbon.absence)

shapiro.test(cp$Carbon.presence)


wilcox.test(cp$Carbon.absence,cp$Carbon.presence)

shapiro.test(cp$pH.absence)

shapiro.test(cp$pH.presence)

t.test(cp$pH.absence,cp$pH.presence)

shapiro.test(cp$Nitrogen.absence)

shapiro.test(cp$Nitrogen.presence)

wilcox.test(cp$Nitrogen.absence,cp$Nitrogen.presence)

install.packages(vegan)

library(vegan)

#diversity indices plotwise

ab=read.csv("thesis Abundance and richness of presence data.csv",header = T,sep = ","
)

Ab

dd <- diversity(ab[,-1],index = "shannon")

Dd
```

```
cd=read.csv("Thesis abundance and richness of absence data.csv", header = T,sep = ",")

Cd

dd<- diversity(cd[,-1],index ="shannon")

Dd

#ggplot2

cp=read.csv( "Data of presence absence for abundance  in R.csv", header = T, sep = ",")

Cp

head(cp)

aaa <-
ggplot()+geom_boxplot(data=cp,aes(x=Plot.no.,y=Abundance))+theme_classic()+
labs(x="Invasion", y="Abundance of macroinvertebrates")+

 theme(legend.position = "top")

Aaa

ccc<-
ggplot()+geom_boxplot(data=cp,aes(x=Plot.no.,y=Species.richness))+theme_classic()+

 labs(x="Invasion", y="Species richness of
macroinvertebrates")+theme(legend.position = "top")

 Ccc

#### correlation test

library(corrplot)

ch=read.csv("amir send files new one.csv", header = T, sep = ",")

names(ch)

ch1<-ch%>%select(Carbon,pH,nitrogen)

correlation<- cor(ch1)

correlation

corrplot(correlation)

summary(correlation)
```