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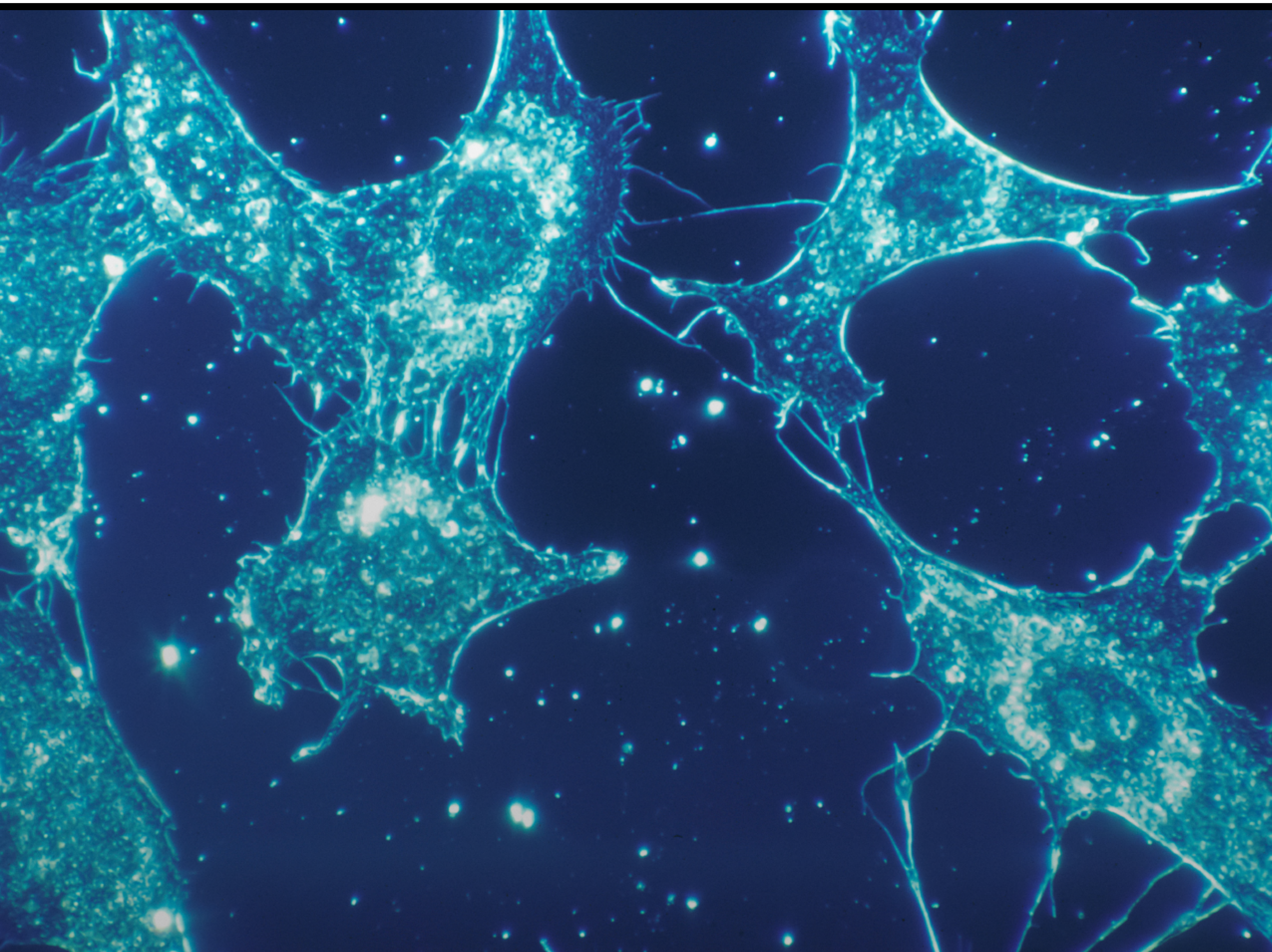

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Looking Ahead While Preserving Heritage: A Review on Medicinal Willow

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Abstract: Willows have been used for millennia for medicinal purposes worldwide. The isolation of salicin in the 18th century from willow bark extracts eventually led to the development of aspirin. Despite the prevalence of aspirin, willow extracts remain popular remedies in countries like Bolivia, Mexico and Italy. Research highlights the benefits of using whole plant extracts, which contain a variety of beneficial compounds beyond salicylic acid, potentially offering synergistic effects and fewer side effects compared to synthetic medications. Recent studies have uncovered the bioactive properties of understudied willow species and the need for innovative extraction methods. As antibiotic resistance becomes a pressing global issue, the exploration of willow extracts for their antibacterial and other medicinal properties, such as anti-inflammatory, antioxidant and anticancer, presents exciting prospects. Future research should focus on characterizing unknown compounds, understanding the mechanisms of action, and promoting the study of tropical species like *Salix humboldtiana*, commonly named as Mexican willow.

Keywords: aspirin, Salix, traditional medicine, willow extract

Introduction

The medicinal use of willows (*Salix* spp., family Salicaceae) has a very long history. At least since the 5th century B.C. there are records of its recommendation by Hippocrates, the father of medicine, who advised the use of willow to reduce fever and relieve pain (Karagiannis 2014). In addition to these traditional therapeutic uses widely known in the West, there are also some little-known uses such as the use of *S. tetrasperma* to treat diabetes in India (Kumar and Janardhana 2012) or *S. aegyptiaca* in Iran for treatment of anemia and vertigo (Asgarpanah 2012). In 18th century, the first steps were taken towards the isolation of the active principle of willow (Hedner and Everts 1998), which culminated in the creation of aspirin presumably by Felix Hoffmann in 1897 (Ugurlucan et al. 2012), although there are doubts as to its authorship (Sneader 2000). According to the World Health Organization, the discovery of aspirin was inspired by traditional medicinal practices that utilized willow bark extracts (World Health Organization 2002).

Currently, traditional and conventional medicine coexist in the world, the former being a fundamental and underestimated part of health care (World Health Organization 2002). Despite the existence and wide diffusion of aspirin, the cure of ailments through willow extracts, especially through the decoction of its leaves or bark, is still in force. In Bolivia, as shown in a survey conducted among people from the mestizo population, it is common to prefer the use of medicinal plants over care by a conventional doctor in the hospital, either because of distrust or economic limitations, with willow –specifically *S. humboldtiana* (Figure 1)– being the main remedy used to combat musculo-skeletal problems (Quiroga et al. 2012). In Mexico, willow is a plant of great cultural importance, and is used to treat “hot and cold diseases” –as they are called in traditional medicine–, fever and headache and to prevent miscarriages (Barrales-Cureño et al. 2022). Idolo et al. (2010) studied the traditional uses of plants in the Italian Apennines, including the role of *S. alba* in treating the flu, and emphasized the importance of species conservation and the creation of protected areas for preserving ethnobotanical knowledge.

There are some advantages to using medicinal willow extracts instead of its isolated active compound. In industry, for example in aquaculture, an important asset to consider of the use of plant extracts over the use of antibiotics is their lower cost (Rangel-López et al. 2020). Willow extracts not only contain salicylic acid, but also a wide range of secondary metabolites with healing potential such as phenolic acids, flavonoids and glycosides (Tawfeek et al. 2021; Table 1). Alkaloids have also been found in the ideal amount:: enough to relax muscles but not so much as to be toxic and harmful for human consumption (Barrales-Cureño et al. 2022). In this sense, one benefit of consuming a decoction of the plant instead of aspirin is to be able to take full advantage of these compounds and not just the salicylic acid. Although the presence of different therapeutic components has been known for many years (Schmid et al. 2001), it has recently been suggested that they may have a synergistic effect on their bioactivity (Tienaho et al. 2021). In addition, medicinal plants are often better assimilated than their synthetic derivatives and have fewer side effects (Piatczak et al. 2020); however, in the case of the therapeutic use of *Salix* extracts, there is concern about allergic reactions in salicylate-sensitive individuals (Le et al. 2021).

The work of Ruzmetov and Ibragimov (2023) shows that during the 21st century there was a growing interest in medicinal willow, which is manifested by an increase in the number of publications on salicylic acid. New lines of research on medicinal willow recurrently question conventional methods of either extraction or cultivation. Antoniadou et al. (2021) suggested that there are doubts about the efficiency of the current extraction method of salicin. Gligoric's team in Serbia was engaged in characterizing the bioactivity of willow extracts using different extraction methods, such as maceration (Gligoric et al. 2019), and recently, with ultrasound-assisted extraction (Gligoric et al. 2023). In terms of cultivation conditions, there is a growing need to focus on willows adapted to local climatic conditions. So research on the bioactive properties of willow bark has been conducted in Finland (Tienaho et al. 2021). Moreover, Salih et al. (2024) have proposed the use of *Salix* crops for energy purposes in that country and the extraction of phytochemicals during the pruning process.

Despite the long history of willow as a medicinal plant, research continues to be conducted and promising and innovative discoveries are being continued to be made. A promising species, *S. eleagnos* has been studied for anti-inflammatory use (Gligoric 2020); and recently, the medicinal potential of *S. schwerinii* and *S. kochiana* has been uncovered, revealing new sources of salicin (Lee et al. 2023).Increased attention is also being paid to the antioxidant potential of willow, having recently been recorded for the first time in *S. alba* leaves (Piatczak et al. 2020), which is an appealing breakthrough as it is a well-studied and widely distributed species. Tienaho et al. (2021) first explored the antifungal and antiyeast potential of extracts of *S. myrsinifolia*, *S. phylicifolia* and some common hybrids from Finland, but have not verified their efficacy. However, they did prove their effectiveness against the non-enveloped enterovirus CVA9, which represents a novelty in the bioactivity studies of willow extracts. In recent years, *Salix babylonica* positioned itself as a promising species to combat common diseases of small mammals (Salem et al. 2017) and trout (Rangel-López et al. 2020).

An interesting approach in the studies of different medicinal willow species is to find unconventional cures for diseases that represent a major public health problem, such as cancer (Hostanska et al. 2007), inflammation due to COVID-19 (Le et al. 2021) and rheumatoid arthitis (Gligoric et al. 2023).Recently, the use of willow extracts to cure bacterial-associated diseases began to be proposed in a context of increasing antibiotic resistance (Salih et al. 2024). The team of Enayat et al. has investigated the potential of *S. aegyptiaca* to inhibit the proliferation of colon cancer cells (Enayat et al. 2013, Enayat & Banerjee 2014) in relation to the anti-inflammatory property, while Ahmad et al. (2023) have focused on the antioxidative properties of *S. mucronata* and have shown encouraging results for the treatment of liver and colorectal cancer. Cancer is a multifactorial and complex disease; therefore, further research is needed, particularly in animal models, before recommending the use of these therapies. It would be interesting to consider the use of willow extracts at least as a complementary therapy to conventional treatment. However, the recommendations of the World Health Organization (2013), regarding some possible risks of the use of traditional therapies such as the quality of the extract, delay in diagnosis, side effects, among others, should be taken into account.

This review has recapitulated some of the main lines of research on medicinal willow in recent decades. As future perspectives, progress can be made in several directions. One interesting line is to continue with the characterization of unknown compounds or to explore the medicinal potential of willow species that have been little studied. The mechanisms underlying the antibiotic activity of *Salix* extracts remain to be studied in depth, although their antibacterial effects have been well documented (González et al. 2020; Wahab et al. 2022; Salih et al. 2024). In relation to the proposal by Tienaho et al. 2021, to investigate willows from different climates, it would be interesting in the future to promote the study of the medicinal properties of *Salix humboldtiana*, the only willow native to South

America and the only one with an almost entirely tropical distribution (Dickmann and Kuzovkina 2014).

In conclusion, more than a century has passed since the first version of aspirin was developed and it is reasonable and desirable to continue researching the plant that gave rise to its active compound. In such a changing context, where new diseases emerge or some that seemed to have been eradicated reappear, it is necessary to keep scientific research dynamic and adaptable. This means betting on interdisciplinary scientific-technical development and recovering ancestral knowledge. There is also a need to think locally, without losing sight of standardized protocols and advances already made on a global scale, in order to include new willow species or new growing conditions in the panorama. Since this review was based on a bibliographic review of articles from indexed scientific journals, advances or new applications of medicinal willow in the field of popular knowledge have probably been omitted. It is often this type of knowledge that advances more quickly, while scientific research takes longer and is not always able to respond to the demands of society. In this era of information overload, the best way to bridge the gap between the two worlds is to adapt research to society's demands for more eco-friendly extraction methods, more integrated medicines that not only have anti-inflammatory properties but also provide other benefits and a more local solution to global problems such as antibiotic resistance.

Conflict of Interest statement: The author declares having no conflicts of interest to disclose regarding this study.

Table 1. Medicinal properties of the main compounds found in *Salix* extracts.

Compound	Properties	References
Flavonoids	Antioxidant, neuroprotective, hepatoprotective	Ramos et al. 2019 Hossain et al. 2009 Sobeh et al. 2019 Wahid et al. 2016
Phenolic glycosides	Anti-inflammatory	Karawya et al. 2010
Organic acids	Antibacterial, anti-inflammatory	Adamczak et al. 2020 Furman 2018
Sterols and terpenes	Anti-inflammatory	Gutiérrez et al. 2017
Simple phenolics	Antibacterial	Masika et al. 2005 Fayaz & Sivakumaar 2014

Figure 1: An adult *Salix humboldtiana*, the only willow species native to South America.

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Antioxidant Effects Following Oral Administration of *Foeniculum vulgare* in Male Rats

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Abstract: Fennel (*Foeniculum vulgare*), a perennial herb belongs to the carrot family and comprises of yellow flowers and feathery leaves. Due to highly fragrance, it is widely used in cooking and is one of the primary components of absinthe. Fennel has become established along roadsides, in pastures, and in various open areas around the world. Present study was carried out to evaluate the antioxidant effects following fennel seeds administration in male albino rats. Twelve albino rats were divided into control and tests. Control was treated with normal saline while treatment was given at concentration of 200 mg/kg/ml of fennel seeds aqueous solution in oral form for three weeks. Antioxidant activities were evaluated by measuring the levels of Malondialdehyde, Catalase, Glutathione peroxidase and Glutathione reductase levels in blood. The administration of fennel seeds significantly enhanced the antioxidant activity showing active action against free radicals in body. It is suggested that fennel seeds can be used as a functional health boosting meal. Further neurochemical research is needed to confirm the mechanism at molecular level.

Keywords: Fennel seeds, Catalase, Malondialdehyde, Glutathione peroxidase and Glutathione reductase

Introduction

Herbs have been used as medicine for a long time; either parts or seeds of plants as phyto-constituents, specifically as medications and other pharmaceutical products. Different herbs like fennel, curcumin, black seeds and cardamom are widely used as medicinal substances (Yang and Shin, 2015). Cytokines involved in inflammatory reactions are produced in a sequential order of events. Additionally, its antioxidant characteristics might reduce stress and stress-related disorders (Koppula & Kumar, 2013). Fennel, Foeniculum vulgare is a medicinal plant which has a significant smell, belongs to the Apiaceae family. It shows properties such as liver protection, blood clot prevention, antioxidant defense, bacterial fighting, inflammation reduction, and fungus inhibition (Rather *et al* 2016). Several macromolecules like carbohydrates, protein, fats, vitamins and minerals are rich in fennel seeds (Bukhari *et al* 2014).

Fennel seeds are found to help reduce neuronal damage by maintaining stable levels of amyloid precursor proteins isoforms and oxidative stress markers (Bhatti *et al* 2018). It has been observed that fennel extract has an anti-cholinesterase property and may help improve memory impairments in Alzheimer's disease and dementia (Joshi & Parle, 2006). Fennel in the diet, along with other dietary spices, can lead to a notable decrease in food transit time, and promote a healthy appetite and digestion. It contains high levels of anethole spread evenly throughout the plant, but mainly concentrated in the seeds (Patel and Srinivasan 2001). The reason for fennel's digestive and carminative effects is due to this compound, while its delightful flavor and unique scent make fennel a tasty vegetable to incorporate into dishes. Vegetable charcoal has been known for its capacity to easily absorb liquid and gases in the intestines, as well as aiding healthy intestinal bacteria that enhance proper digestion (Patel and Srinivasan 2001; Noreen *et al* 2023). Some scientists reported that fennel could remove gas from the digestive system, releasing the respiratory system and providing a soothing impact on respiratory disorders; anethole and fenchone, the key components of its essential oil, have been proven to have a mucous-eliminating effect on the respiratory system (Noreen *et al* 2023). Of medicine, fennel seeds are boiled in syrups to alleviate cough and breathing difficulties (Razieh *et al* 2022). Fennel essential oil is considered beneficial for treating obesity (Hossein *et al* 2016). The weight loss effects of fennel are thought to be due to appetite suppression. Anetholes found in fennels could possibly be utilized for the prevention and treatment of cancer (Anand *et al.*, 2008). Anethole can disrupt TNF signaling, causing activation of necrosis factors including NF-κB and programmed cell death. Researchers explained that anethole might inhibit NF-κB-dependent gene expression triggered by TNF, which regulates the expression of certain genes related to cancer development and inflammation according to (Chainy *et al.*, 2000).

It has been observed that plants that mostly contain hydroxyl groups and isoprene units in their structure contain antioxidants properties (Manzoor *et al.* 2022; Süntar 2020). The fennel fruits yield products are important and contain antioxidant properties (Mata *et al.*, 2007). Previous studies showed that flavonoids present in fennel plants, recognized for their ability to combat free radicals with antioxidant properties (Anwar *et al* 2009). It has been well documented that significant amounts of overall phenolic compounds flavonoids present in plant have a capacity of radical scavenging that can inhibit peroxidation. It has been reported that reactive oxygen species (ROS) can be generated in the skin through chemical ionization and/or UV radiation, as well as enzymatically by polymorphonuclear leukocytes at infection sites (Goto *et al 2002).* Furthermore, it has been found that fennel seeds enhance catalase activities and increase the levels of plasma superoxide dismutase, along with an increase in high density lipoprotein-cholesterol level while the level of malondialdehyde as an indicator of lipid peroxidation was notably reduced in fennel treated individuals as compared to control (Eun and Hwang 2004).

The present study was designed to find out the antioxidant properties of fennel in albino rats especially to know the mechanism of action of fennel against reactive oxygen species that are commonly incorporated with environment which could elaborate about fennel and its beneficial effects in the development of various medications that are essential for preserving human health.

Materials and Methods

Fennel seeds used in current study are locally purchased. Fennel seeds known amount 200mg soaked in water overnight, and the extract was collected which was used as dose given to the rats following the protocol of Sajid et al (2017). Twelve locally bred Albino Wister rats (150-180g) purchased from Dow University Ojha Campus. All animals were kept individually and provided standard conditions with free access to a rodent diet. Animals were divided into two groups control and test. Control was given saline while test animals were given fennel seeds extract and administered orally for 23 days and were sacrificed at 25th day. Blood was collected in heparinized tubes to obtain plasma and immediately was stored for antioxidant analysis. All experiments were conducted according to a protocol approved by the Institutional Ethical Care Committee.

Determination of Antioxidant Enzyme Activities

Estimation of activity of catalase, malondialdehyde, glutathione peroxidase and glutathione was performed following the protocol as described by Sajid et al (2017).

Data Analysis

The data was analyzed using by Student's *t-test*. The results are represented as mean ± S.D, and significant values are indicated when $p<0.05$.

Results

The effect of fennel extract on catalase antioxidant enzyme is shown in Fig. 1. Data analyzed by the t-Test reveals the significant effect on administration of fennel extract significantly increased (t=-5.61, df=10) catalase enzyme as compared to control. Malondialdehyde (MDA) is a marker of lipid peroxidation showing oxidative stress and redox signaling studies. A significant decrease was found (t=1.45, df=10) under the administration of MDA when content LPO had a significant effect (Fig. 2). The current findings showed that fennel administration could be beneficial for membrane structure and helps in more protection against oxidative damage by free radicles. Similarly, Glutathione Peroxidase; an intracellular antioxidant enzyme was found significantly increased (t=-2.17, df=10) as compared to control (Fig. 3). Glutathione reductase enzyme was found non-significantly increased (t=-0.42, df =10) as compared to control (Fig. 4).

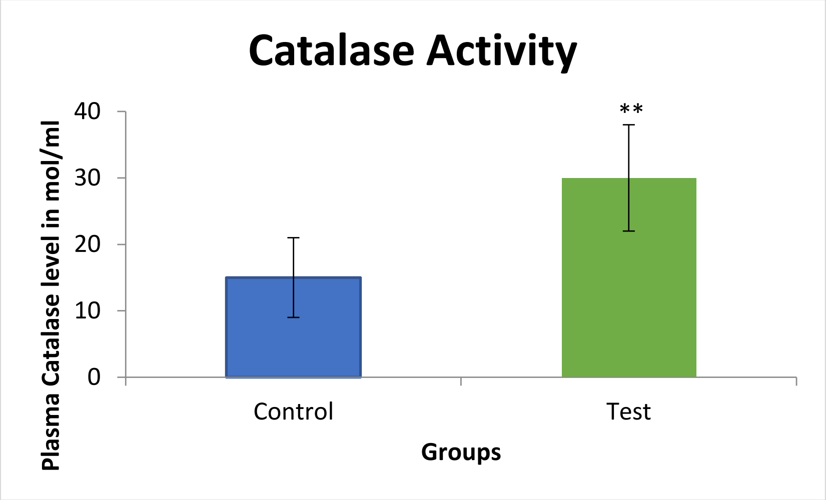

Figure 1: **Catalase enzyme activity in Blood Plasma**
Mean ± SD (n=6) analysis by student's t-test revealed a significant effect (**P<0.01vs control)

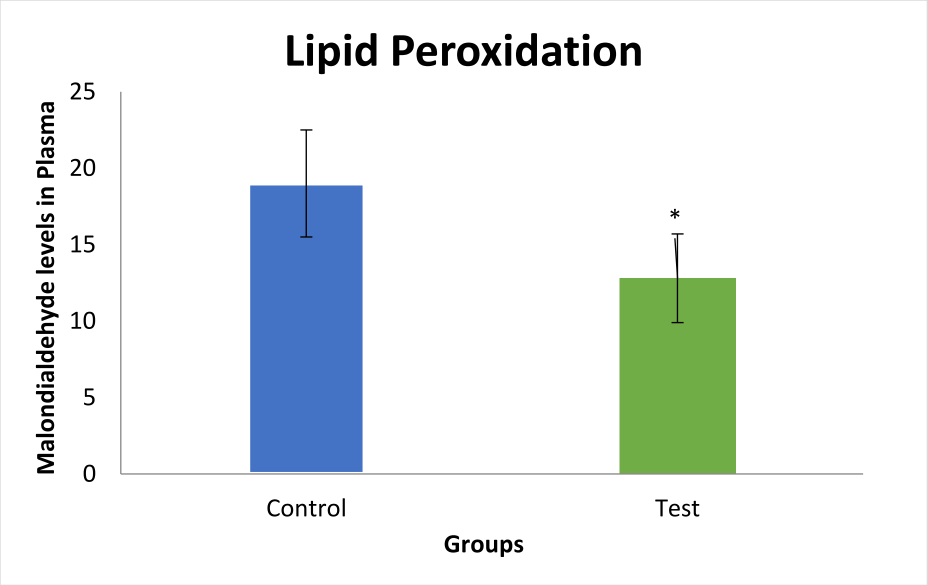

Figure 2: **Malondialdehyde (MDA) levels in Blood Plasma**
Mean ± SD (n=6) analysis by student's t-test revealed a significant effect (*P<0.05vs control).

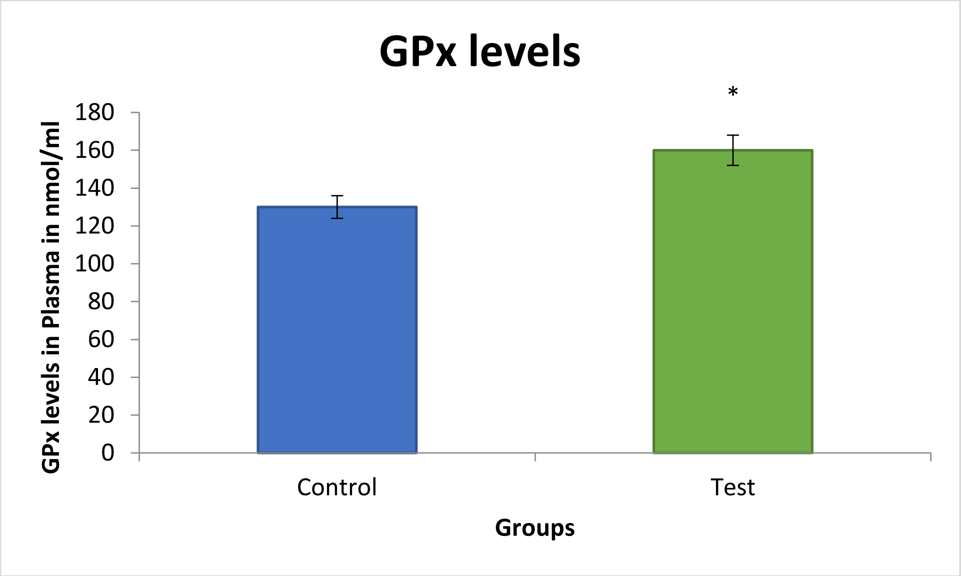

Figure 3: Peroxidase Enzyme levels in Blood plasma
Mean ± SD (n=6) analysis by student's t-test revealed a significant effect (*P<0.05vs control)

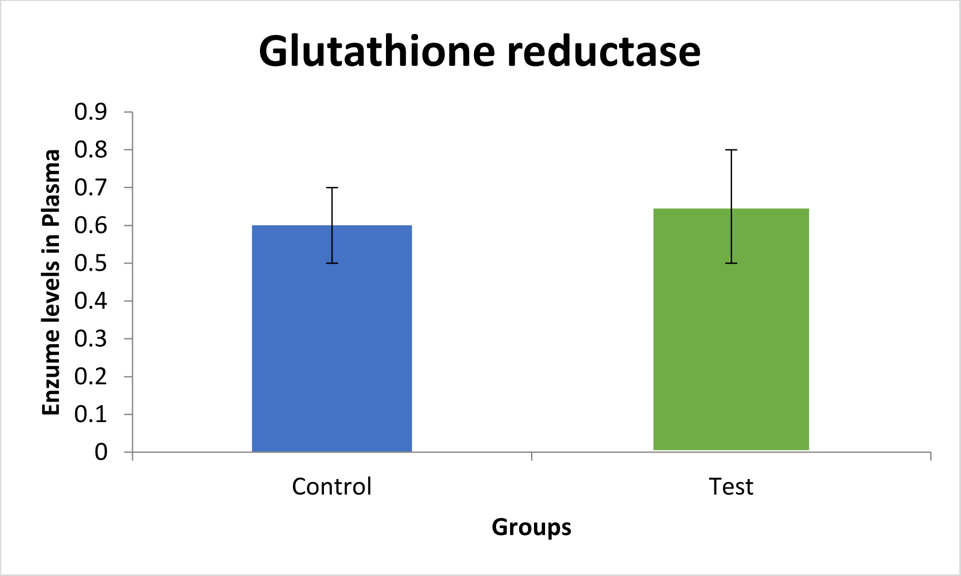

Figure 4: Reductase levels in Blood Plasma
Mean ± SD (n=6) analysis by student's t-test revealed a non-significant effect (P>0.05vs control)

Discussion

The current study revealed that fennel seeds have a notable effect on catalase, MDA content, and glutathione peroxidase, while showed no significant effect on glutathione reductase.

Studies reported that free radicals are reactive oxygen species (ROS) that can damage cells and are responsible for many diseases infertility, different carcinomas, diabetes, and cardiovascular issues, illness, getting older, and neurodegenerative diseases (Mata *et al.*, 2007). The fennel fruits yield essential oil, water, and ethanol extracts that are important and contain antioxidant properties (Noreen *et al.*, 2023). Furthermore, it has also been reported that water and ethanol extracts of fennel show 99.1% and 77.5% peroxidation inhibiting in the linoleic acid system, respectively and α-tocopherol, a natural antioxidant (Ahmed *et al* 2019). Both samples demonstrate strong abilities in scavenging free radicals, superoxide anion radicals, hydrogen peroxide, and chelating metals, all of which are closely related (Oktay *et al* 2003). The level of concentration suggests that fennel seeds have the possibility to be a source of antioxidants that occur naturally. The present study showed significant effects of fennel on antioxidant enzyme levels that can be attributed with the beneficial and protective effect of seeds as reported in different previous studies to assess the antioxidant activity of fennel seeds by administering in rats 200 mg/kg/day. Previously it has been shown with a significant effect on SOD and catalase levels, while lipid peroxidation was significantly decreased (Hassan *et al* 2023). Effects on antioxidant enzymes levels and could be attributed to a decrease in harmful impact from the buildup of oxygen radicals that might produce positive impact on pathological changes, particularly in inflammatory conditions. Hassan *et al* (2023) noted an increase in HDL-cholesterol levels in fennel seeds administered group. This HDL effect could be occurring because it inhibits the oxidative alteration of LDL. Ragaa *et al* (2011) identified that fennel seed extract effectiveness in albino mice for its protective mechanism in cell and as a non-toxic radioprotector. After administration of fennel seeds extract, the improvement has been noticed in liver and tumor tissue of mice with ascites carcinoma due to notable rise in MDA levels and reduction in catalase activity and glutathione Consistently the current findings showed a decrease in MDA participate in maintaining integrity of membranes of cell. In recent study glutathione peroxidase has crucial effect so that the oxidative stress can be reduced by scavenging of free radicals and conversion of hydrogen peroxide radical into water or alcohols fennel seeds contain these natural antioxidants in large quantity. Due to its high contents of polyphenols and flavonoids, this plant can help in inhibiting the production of free radicals (Parejo *et al.*, 2004b). In the body different antioxidants like molecules are involved that can neutralize reactive molecules. Moreover, Sura *et al* (2024) showed a notable increase in Glutathione peroxidase levels in chicks that consumed fennel seeds, along with a significant decrease in malondialdehyde; these alterations may be attributed to fennel's effective radical scavenging properties. A notable increase in the functions of antioxidant enzymes was seen in diets that included fennel (Nickavar and Abolhasani, 2009). Furthermore, Rezq (2013) indicated in past research that providing diets enriched with varying amounts of fennel seeds markedly enhanced the serum levels of Superoxide dismutase (SOD) and Glutathione peroxidase enzyme relative to the control group. Furthermore, it has been reported that dieting chicks with a high-fat diet enriched with fennel seeds notably lowered serum MDA levels in comparison to the positive control (Rezq, 2013). Flavonoid and phenolic compounds contain antioxidants with a radical scavenging mechanism, which can potentially change the physiological antioxidant status by increasing GSH-Px and decreasing malondialdehyde, according to current findings (Zayachkivska *et al.*, 2005). The current results also explained beneficial effects of fennel seeds administration as enhancing activities of antioxidant levels in blood sample (Mohamad *et al* 2011). They further concluded in their research that fennel seed extract might diminish oxidative stress and safeguard mouse cells from harm induced by reactive oxygen species, and it may serve as a secure, efficient, and readily available source of natural antioxidants to enhance the oxidative stability of fatty foods while in storage. Extracts possess an antitumor effect by influencing lipid peroxidation and enhancing the antioxidant defense system in EAC-bearing mice, whether they were exposed to radiation or not. Mohammad *et al.* (2011) found that the activities of superoxide dismutase and catalase and MDA and GSH were enhanced. The current study in consistence to the findings of Choi *et al* (2004) revealed a decrease in MDA contents. Body has its own antioxidant defenses to keep free radicals in check and current study provides a concept that fennel seeds strengthen defense system of body.

Conclusion

Present research showed that fennel seeds serve as excellent sources of vital phytonutrients and phytochemicals as fennel seeds have significant effect on catalase, MDA and glutathione peroxidase levels. Fennel seeds, thus, can be used as a functional, health boosting meal. Synthetic drugs possess several side effects that could be harmful for body, so knowledge of using natural plants as an alternative medicine for diseases has been raised. However, the use of fennel seeds as natural medicine for many years has been acknowledged. Further neurochemical research is needed to confirm the mechanism of fennel at molecular level.

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Distribution, Diversity, and Ecological Traits of Earthworms in Different Habitats: Implications for Conservation and Management Practices

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Abstract: The present study investigated the distribution and diversity of earthworms in five different habitats, comprising grassland, agricultural fields, forests, wetlands, and plant-associated soil in Jammu region, Jammu and Kashmir, India. The study analyzed various environmental factors to understand their influence on the abundance and diversity of earthworms, which notably observed silty clay loam texture for forest, grasslands, and plant-associated habitat sites, whereas silt loam and silty clay were characteristic of wetland habitat and agricultural fields, respectively. The soil pH and organic carbon were highest in plant-associated soil and lowest observed in wetland habitat site. The agricultural field recorded the highest percentage of available nitrogen and the lowest observed in wetland. The findings revealed the distribution of 36 different types of genus/species across all habitats in which grassland habitats exhibited the highest abundance of earthworms, with 258 numbers followed by agricultural fields with 255, forests with 137, wetlands with 75, and plant-associated habitats with 72. Furthermore, the study demonstrated that all the earthworm species were significantly influenced by specific environmental factors in their respective habitats. However, in environments like agricultural fields and grasslands, soil parameters had minimal impact on species abundance. The study also identified the dominance of different ecological traits across habitats, highlighting the importance of morphometric traits in understanding the ecological function of earthworms in different habitats. Overall, the results could have practical implications for conservation and management practices in these ecosystems, providing insights into the distribution and diversity of earthworms in different habitats and their relationship with environmental factors.

Keywords: Earthworm, Diversity, Habitats, Environmental factors, Functional traits

Introduction

Earthworms are the major dominant decomposer group community and play a significant role in ecosystem functioning through ingestion, respiration, and egestion (Edwards & Arancon, 2022; Tagliabue et al., 2023). Their feeding and burrowing behavior increases the surface area of organic content, which helps to convert and promote vertical transport of organic matters in soils (Capowiez et al., 2021). They are known as "ecosystem engineers" due to their ability to substantially change the physical and chemical properties of their soil environment (Zhang et al., 2023). They accomplish this by consuming large amounts of dead plant material, which they break down in their digestive systems and excrete as nutrient-rich castings. These castings enhance soil fertility and structure, promoting plant growth and nutrient cycling (Reyes et al., 2023). Additionally, earthworms enhance soil health and productivity by burrowing through the soil, which improves soil aeration, water infiltration and plant root penetration and growth (Bayon et al., 2021). Earthworms distribute organic matter and essential nutrients like carbon and nitrogen throughout the soil. This process enhances soil health and fertility, and promotes biodiversity by creating various habitats for different organisms (Edwards & Arancon, 2022a; Fonte et al., 2023). As they bring all these changes in the soil ecologist referred them as the biological indicators of soil quality (Ansari & Ismail, 2012; Fusaro et al., 2018). Earthworms are classified into three main ecological categories based on their feeding and burrowing strategies (S et al., 2016). Epigeic earthworms, characterized by their small, cylindrical bodies, live on the soil surface and in leaf litter, where they consume decomposing plant material and create shallow burrows. Anecic earthworms, with their larger and more robust bodies, live in deeper soil layers, creating vertical burrows up to several meters deep, where they transport and consume surface organic matter. Endogeic earthworms, which have smaller bodies than epigeic and anecic earthworms, live and feed within the soil, creating horizontal burrows at the soil-litter interface, consuming, and mixing soil, organic matter, and mineral particles.

To assess the impact of earthworms on ecosystem services, ecological classification has been used to link their morphological traits with their ecological functions (Bottinelli & Capowiez, 2021;Walia & Kaur, 2024). Traditional taxonomy-based methods may not be sufficient to explain the diverse roles of earthworms in ecosystem functioning, therefore incorporating functional analysis was recommended (Andriuzzi et al., 2016). Earthworm species in functional groups share several morphological traits, and use of these traits may provide additional information on changes in biodiversity and facilitate better comparison with other geographical regions. Functional traits or attributes are the quantitative traits at individual level such as morphological, physiological, phenological or behavioral features, which defines the organisms with respect to its ecological roles (McGill et al., 2006; Díaz et al., 2013). Linking taxonomic framework and functional trait approach could be more effective to explain heterogeneity in community assembly and interspecific effects on ecological processes (Funk et al., 2017). Generally functional traits analysis was used to investigate species abundance and distribution across environmental gradients (Bernhardt-Römermann et al., 2011;Violle et al., 2011).

Traits can vary substantially among individuals of a given species, during growth for example, since adults are often 15-40 times larger than newly hatched individuals (Lavelle, 1978). Additionally, it can shed light on relationships between community structure and ecosystem processes and the impacts of climate change on species range shifts.

Hence, a functional trait framework is now regarded as a promising way of revealing generalities in species distribution, community assemblages and ecosystem processes (McGill et al., 2006;Violle et al., 2007). In this study we considered earthworm traits that are expected to influence soil processes and measured the values or forms taken by these traits, (Violle et al., 2007). The traits selected influence the ability to burrow and bioturbated (an effect trait) or, like pigmentation (likely a response trait), to survive in the litter and the surface environment.

We examined the earthworm community structure along the different habitat gradient using traditional diversity measures, taxonomic properties, and the functional group concept based on biological traits to answer the following questions:

Does the earthworm community structure and functionality change as a result of different heterogeneous habitat gradient.

What are the trends and causes of variation in the structural and functional diversity of earthworm community throughout the different habitats of Jammu regions; specifically, are the soil parameters the primary cause or are other factors (such as sediment, organic carbon, etc.) more significant?

Materials and method

Study area

The study was conducted in the Jammu region of Jammu and Kashmir, India, in varied range of habitats including Agricultural field (Old Satwari), Grassland (Chatha farm), Forest (Sitni Nagrota), Wetland (Gharana wetland) and plant associated (IIIM field). These habitats vary in their ecological characteristics and provide unique environments for different species for survival. Jammu is located at coordinates 32.7059° N latitude and 74.8798° E longitude, indicating its precise geographical position. The temperature ranges widely, fluctuating between 10-15°C during cooler periods and rising to 40-45°C during the hotter months and the annual precipitation received in the region is approximately 1332 mm.

Earthworm sampling

Earthworm specimens were collected by handpicking and digging methods from five different habitats on a monthly basis. The sampling strategy followed by selecting three stations from each habitat and each station selected in a range of 100-400 meters apart. However, the distance between each habitat sites varies from 2-5 km. The collected specimens were put in the polybags labelled with habitat site and the date of collection. In the laboratory, earthworms were sorted from monoliths, rinsed with tap-water followed by distilled water and gently dried with paper napkins. Earthworms were then anesthetized with 20 % ethyl alcohol and identified them by following the available taxonomic keys (Stephenson, 1987; Julka, 1988). All adult specimens were grouped by its morphology and identified at species/genus level when possible.

Soil parameters

A differential GPS was used to measure the elevation and geographic coordinates of the sample locations at the centre of the plots. Soil samples were also collected from all habitat sites and physico-chemical parameters were measured.

According to Baize and Jabiol's key, the coarse material size distribution of the top 5 cm of soils was visually evaluated on-site using gravels of various sizes (big > 5 cm, medium > 2 cm, and small) following the key of Baize and Jabiol (1995). Soil texture was measured by Bouyoucos hydrometer method (Bouyoucos, 1962), For organic carbon (OC), and available nitrogen (N) measurements, soil samples were extracted at each sampling site, homogenised and sieved at 2 mm, and measured following Chromic acid Digestion (Walkley & Black, 1934) and Alkaline permanganate method (Subbiah & Asija, 1956). Electric conductivity (EC) and pH was measured by suspension method (1:2.5) (Jackson, 1967). Bulk density was estimated by following the Blake and Hartge method (Blake & Hartge, 1986). Additionally, the meteorological information, such as temperature, rainfall and humidity of all sampling months was recorded to understand more insight into the environment on the distribution pattern of earthworms in varied ecosystems.

Functional traits and its attributes

We have measured five traits (Body length, Anterior musculature (AM), Body Pigmentation, Ecological category, and setae shape) of all the earthworms and each trait was linked with certain specific ecological function. Body length was associated with the overall strength and divided into two categories 10-15cm and above 15 cm. Length was measured by fixed the organisms in formalin. Anterior musculature (AM) is also an indicator of burrowing ability and is well developed in deep burrowing earthworms. The difference between the body diameter prior to the clitellum, where the AM is present, and posterior to it, when no specific muscles are present, was used to determine the relative strength of the AM. Based on the relative strength of AM we divided into three categories as poorly developed AM (0 mm), moderately developed AM (0-4 mm) and well-developed AM (Above 4 mm). Body pigmentation facilitates camouflage which provides protection from predators. The body was categorized into three categories as uniformly pigmented, dorsally pigmented, and non-pigmented based on which earthworm performed different ecological strategies. Earthworm mainly possessed three distinct ecological categories (epigeic, anecic and endogeic) which form a kind of ecological niche to earthworms. In our study we selected the three main categories to understand its ecosystem functioning. Setae are the chitinous structures which help the organism to form grip with soil while moving. We selected three categories of degrees of development of setae as not visible setae, curved setae, and straight setae.

Statistical analysis

The Bray-Curtis dissimilarity (standardised, square-root transformed) (Bray & Curtis, 1957), based on the relative abundances of earthworm genera, and ordination using the Jaccard similarity index, based on presence or absence, were the two types of similarity measures used in the species-level similarity analysis (Clarke, 1993). Non-metric multidimensional scaling (nMDS) plots were used to display the differences between the samples. To determine the statistical significance of differences in pairwise comparisons of earthworm populations from various habitats, we used a permutational multivariate analysis of variance (PERMANOVA) with two factors: "station" (all stations in the habitat combined) and "zones" (habitats) (M. J. Anderson, 2005; M. Anderson, 2008).

In order to show diversity, the expected number of species in a sample, various diversity indices were determined by using the Shannon-Wiener index (H0) (Weaver, 1963) for species diversity by using natural logarithm (loge), Pielou's index (Pielou, 1966) for species evenness (J0), and Margalef's index (Margalef, 1968) for species richness (d). Principal component analysis (PCA) was then used to identify the geographical patterns based on environmental data using environmental variables. It was created a lower triangular ordination related Euclidean distance matrix (Clarke & Green, 1988). The data were examined for uniform distribution and normalised (by subtracting the mean and dividing by the standard deviation, for each variable) before analysis in order to prepare them for the creation of the Euclidean distance resemblance matrix. If the distribution of the residuals was skewed, the response variable underwent a natural logarithm modification until the best model's assumptions were satisfied. The biota environment (BIOENV) procedure (Clarke & Ainsworth, 1993), which computes rank correlations between a similarity matrix derived from biological data and matrices derived from the environmental variables, was used to examine the relationships of taxonomic and functional traits with environmental variables. This procedure defines a set of variables that "best explain" the biotic structure. To determine which set of environmental factors predicted the multivariate variance in earthworm species assemblages, we used RELATE and a stepwise distance-based linear model permutation test DistLM; (McArdle & Anderson, 2001). To enable the best explanatory environmental variables to be fitted into the model, the adjusted R2 was employed as a selection criterion. All DistLM techniques employed the Euclidean distance as their similarity matrix. An analysis of distance-based redundancy (dbRDA) was used to visualise the results (M. Anderson, 2008).

Furthermore, we used multi-level pattern analysis (De Cáceres et al., 2010) in the R environment (R Development Core Team, 2015) with the "indicspecies" function to perform the Indicator Species Analysis, or IndVal (Dufrêne & Legendre, 1997) to identify the species that would characterise the habitats compared. Monte Carlo randomizations with 1000 permutations were used to examine the statistical significance of the connection between the species and site. Dufrene and Legendre outline the specifics of the procedure (1997). The PERMANOVA+ module of the PRIMER v6 software and the R Development Core Team's (2015) and Dimitriadou et al., (2008) (Dimitriadou et al., 2008) software's procedures were used for all of the studies (Clarke & Gorley, 2006). The taxonomic and functional data set was used to create a schematic diagram that showed the pattern in the various habitats of the Jammu region.

Results

Earthworm collection

A comprehensive sampling of earthworms was conducted in Jammu regions from October 2020- September 2021 in five distint terrestrial ecosystems, including agricultural fields, grassland, forest and wetland ecosystems. Inclusively across all the habitats, the distribution of 36 different types of genus/species was observed, and their numbers varied in all habitats.

Environmental parameters

Soil parameters estimated of all the selected habitats, including agricultural fields, forests, Grasslands, Plant-associated, Wetland ecosystems. The agricultural field recorded the highest clay (46.66%) and available nitrogen (175.62 kg/hac), whereas the wetland habitat reorded the lowest clay (6.66%), bulk density (1.43 Mg m^{-3}), and available nitrogen (62.72 kg/hac). Plant associated soil has the highest pH (7.75) and organic carbon (2.36%), whereas the wetland habitat has the lowest pH (6.3) and organic carbon (0.09%). Similarly, Forest habitat soil estimated the highest electrical conductivity (0.71 dS/m), whereas grassland soil observed the highest sand contents (30%). The bulk density values range from 1.19 Mg m^{-3} for plant-associated habitat to 1.44 Mg m^{-3} for grassland habitat (Table 1). The soil texture varied, with silty clay loam being typical of grassland, plant-associated soil, and forest soil, whereas silt loam and silty clay were characteristic of wetland habitat and agricultural fields, respectively.

Table 1. Soil parameter analysis of all distinct terrestrial habitat sites

Soil parameters	Habitats				
	Grassland	Agricultural field	Forest	Wetland	Plant associ-ated
Latitude	32.6838°N	32.6624°N	32.8119°N	32.7983° N	32.7387°N
Longitude	74.8244° E	74.8302°E	74.9094° E	74.9152° E	74.8525° E
Sand %	30	26.66	14	26.65	30
Silt %	34.66	26	53.33	66.66	34.66
Clay %	33.33	46.66	32.66	6.66	35.33
pH	6.8	7.07	6.4	6.3	7.75
Ec (dS/m)	0.49	0.54	0.71	0.47	0.68
O.C. (%)	0.68	1.14	0.59	0.09	2.36
Available N (kg/ha)	125.44	175.62	137.98	62.72	112.9
B.D. (Mg m-3)	1.44	1.35	1.29	1.43	1.19

Texture Class	Silty clay loam	Silty clay	Silty clay loam	Silt loam	Silty clay loam

PCA ordination plot was constructed on five environmental parameters (Soil texture, pH, Organic carbon, Available N, Bulk density, electrical conductivity (EC)) that influencing the ecological traits showed 92% of variability in the data by PC1 and remaining was by PC2.

Results of the DistLM based on the dbRDA plot are displayed in Fig 2 along with information on species abundance and soil parameter values. Sand plays a significant role in the forest site and bulk density in the wetland, according to the vectors of the environmental variables that the DistLM procedure retained as best explaining the model, whereas available N, clay, and organic carbon (OC) played a significant role in the plant-associated habitat site. However, in environments like agricultural fields and grasslands, the soil parameters had little effect on the species abundance (Fig.1 & 2).

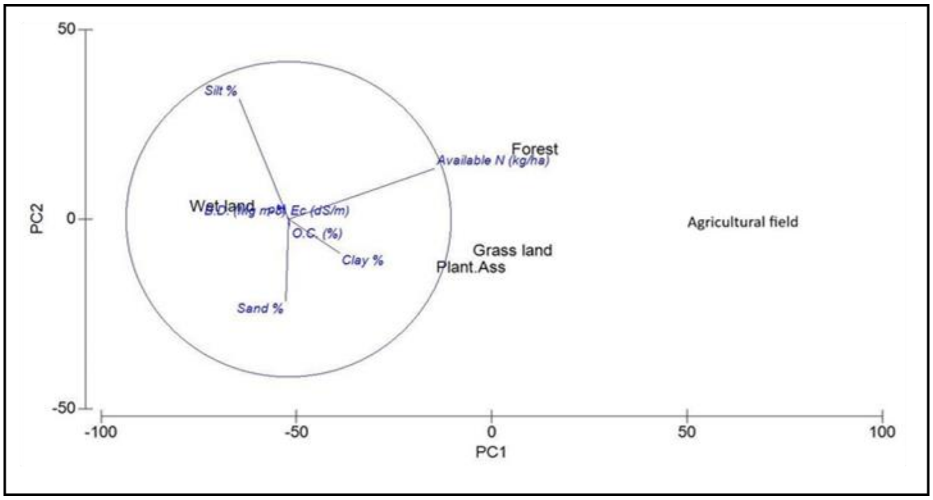


Figure 1: Principal-component analysis (PCA) derived from the contribution of soil parameters in all habitats zone. PCA plot accounted 92 % of total variation by PC1

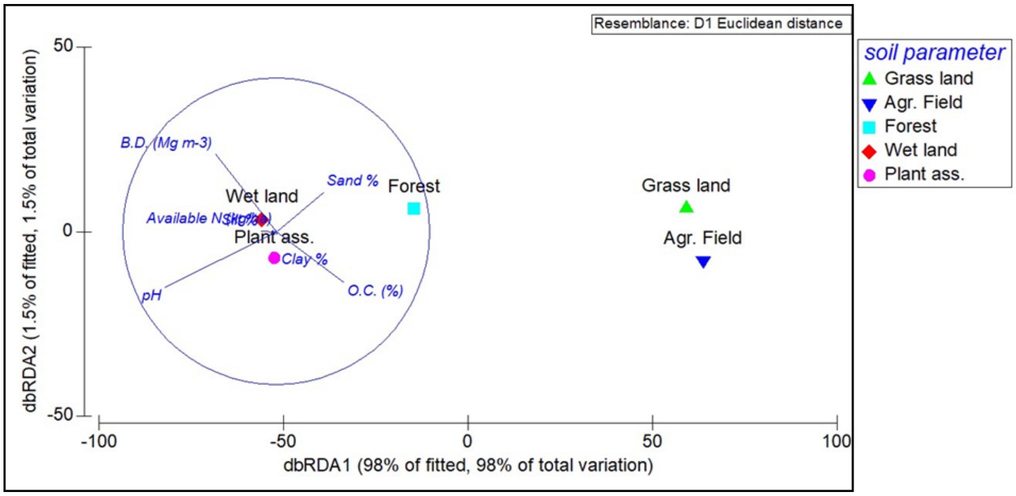


Figure 2: Distance-based redundancy (dbRDA) bubble plot illustrating the DistLM model based on the species assemblage data and fitted environmental variables.

To gain more insight, the meteorological information revealed that the highest temperature was observed in June, while the highest humidity and rainfall were recorded in August and July respectively (Table 2).

Table 2. Meteorological data of all the sampling months

Meteorological information	**Sampling months**											
	Oct-20	**Nov-20**	**Dec-20**	**Jan-21**	**Feb-21**	**Mar-21**	**Apr-21**	**May-21**	**Jun-21**	**Jul-21**	**Aug-21**	**Sep-21**
Avg. Temperature °C (°F)	21.6 °C (70.8) °F	16.4 °C (61.6) °F	12 °C (53.6) °F	10.5 °C (50.8) °F	13 °C (55.4) °F	17.7 °C (63.9) °F	23.9 °C (75) °F	29.1 °C (84.3) °F	30.7 °C (87.3) °F	28.4 °C (83.2) °F	27.3 °C (81.1) °F	25.6 °C (78.1) °F
Min. Temperature °C (°F)	14.6 °C (58.3) °F	9.6 °C (49.3) °F	5.4 °C (41.7) °F	4 °C (39.3) °F	6.2 °C (43.1) °F	10.1 °C (50.2) °F	15.5 °C (59.9) °F	20.3 °C (68.6) °F	23.5 °C (74.3) °F	24.4 °C (76) °F	23.7 °C (74.7) °F	20.7 °C (69.2) °F
Max. Temperature °C (°F)	28.8 °C (83.8) °F	23.8 °C (74.8) °F	19.1 °C (66.3) °F	17 °C (62.6) °F	19.6 °C (67.3) °F	25 °C (77) °F	31.6 °C (88.8) °F	36.5 °C (97.7) °F	36.7 °C (98.1) °F	32.4 °C (90.4) °F	31 °C (87.9) °F	30.5 °C (87) °F
Rainfall (mm)	17	25	46	74	129	113	71	29	108	321	265	115
Humidity (%)	62%	62%	66%	70%	67%	58%	41%	33%	43%	74%	81%	76%

Diversity indices and species richness

A total of 36 earthworm genus/species belonging to different families were found across all the habitats. The highest diversity and abundance of earthworms were observed in grassland habitats, followed by agricultural fields, forests, wetlands, and plant-associated habitats (Table 3). The most dominant species found across all habitats was *Eisenia fetida* with the density of 72 ind. per m^2 followed by *Bimastos rosea* with the density of 36 ind. Per m^2 and *Lampito mauritii* with the density of 35 ind. Per m^2. Some species, including *Millsonia anomala*, were exclusively found in single habitat, such grasslands, whereas *Dichogaster saliens, Metaphire posthuma, Octolasion lacteum,* and *Pellogaster bengalensis* were found in both agriculture areas and grassland habitats. Similarly, *Allolobophora parva* and *Amynthas diffringens* were only found in agriculture fields and forests, however, no restricted species were found in wetlands or plant-associated habitats.

Table 3. Earthworm abundance data collected across five different habitats revealing grassland exhibited the highest richness in terms of numbers

Serial No.	Species numbers	Habitats				
		Grassland	Agricultural field	Forest	Wetland	Plant associated
1	*Allolobophora parva*	0	7	4	0	0
2	*Amynthas diffringens*	0	9	3	0	0
3	*Amynthas morrisi*	11	9	4	3	4
4	*Amynthas spp.*	13	0	3	3	0
5	*Aporrectodea caliginosa*	0	8	4	5	0
6	*Aporrectodea rosea*	9	6	7	8	6
7	*Dendrobaena octaedra*	10	7	8	5	0
8	*Dichogaster bolaui*	0	8	5	0	7
9	*Dichogaster saliens*	11	8	0	0	0
10	*Drawida calebi*	9	4	6	6	4
11	*Drawida ghilarovi*	0	0	5	3	4
12	*Drawida willsi*	4	9	0	0	3
13	*Eisenia fetida*	21	23	9	9	10
14	*Eudrilus eugeniae*	8	10	3	0	3
15	*Eutyphoeus incommodus*	0	10	9	5	0
16	*Eutyphoeus nicholsoni*	12	10	5	0	0
17	*Eutyphoeus sp.*	9	3	0	0	4
18	*Eutyphoeus waltoni*	11	7	0	4	5
19	*Lampito mauritii*	8	11	6	7	3
20	*Lennogaster pusillus*	10	12	0	5	6
21	*Lumbricus rubellus*	7	6	0	0	5
22	*Lumbricus terrestris*	8	6	12	4	0
23	*Metaphire posthuma*	9	8	0	0	0
24	*Millsonia anomala*	9	0	0	0	0
25	*Octochaetona beatrix*	8	9	4	3	2
26	*Octochaetona serrate*	8	9	5	0	0
27	*Octochaetona surensis*	3	6	6	0	0
28	*Octolasion lacteum*	11	2	0	0	0
29	*Pellogaster bengalensis*	9	6	0	0	0
30	*Perionyx excavates*	7	7	4	0	0
31	*Perionyx gravely*	6	5	5	0	2
32	*Perionyx sansibaricus*	7	5	0	5	0
33	*Pheretima alexandri*	7	4	6	0	0
34	*Polypheretima elongata*	10	13	10	0	0
35	*Pontodrilus bermudensis*	3	8	4	0	4
36	*Pontoscolex corethrurus*	5	6	9	4	4

As per the Bray–Curtis nMDS similarity index of earthworm abundance and presence/absence data, it clearly illustrates that all habitats were differ to each other; the explored habiats were 40% dissimilar to each other, except for agriculture fields and forests, which were 30% dissimilar in terms of their taxonomic abundance however, agriculture field and grassland habitats were resembled 80% to each other with respect to species abundance and presence/absence of species (fig. 3 & 4). *Amynthas sp* had the highest contribution to similarity within agriculture field and grassland habitats with 10.07% similarly *D. saliens, E. waltoni* and *O. lacteum* had the highest contribution to similarity within grassland and forest with 5.6% and the mean dissimilarity value was 40.38%. In agriculture field and forest the SIMPER analysis showed that *L. pusillus* was the highest contribution to similarity with the value of 7.69% and the average dissimilarity value was 30.04%. Species *E. nicholsoni* had the highest value of similarity within grassland and wetland habitats with 5.36% and the average dissimilarity value between them was 52.86%, in agriculture field and wetland *P elongate* showed the highest contribution value to similarity with 5.61% and the average dissimilarity value was 51.11%. In habitats forest and wetland *P. elongata* showed the highest contribution to similarity with the value of 8.23% and the mean dissimilarity value between the habitats was 40.74% whereas *Amynthas sps* had the highest similarity contribution value of 6.01% within grassland and plant associated habitats and the mean dissimilarity value was 50.54%.

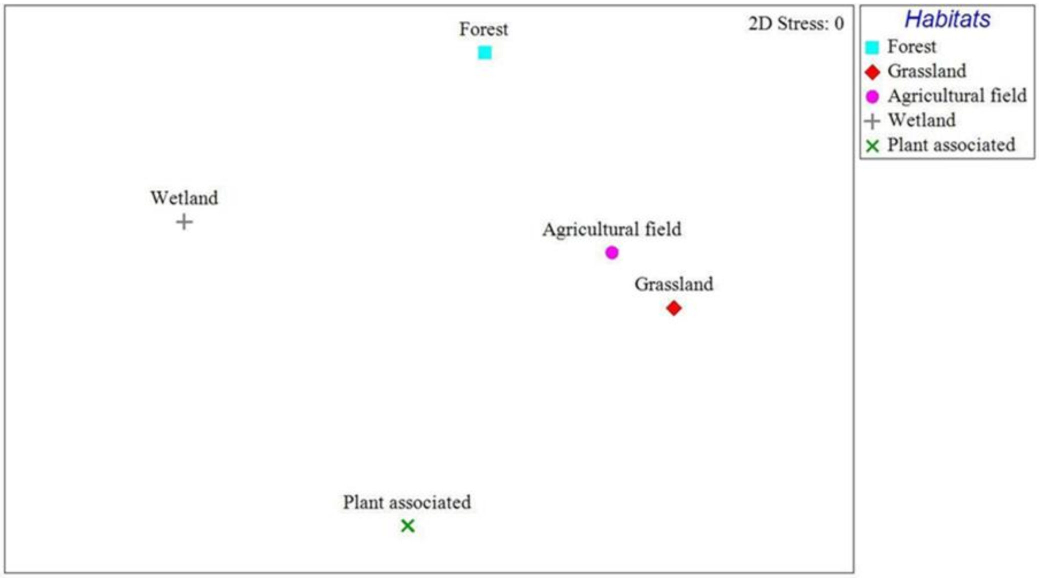


Figure 3: nMDS ordination based on earthworm species presence or absence according to the Bray-Curtis similarity index.

Figure 4: nMDS ordination-based Bray-Curtis similarity index to estimate the degree of similarity in species abundance

The SIMPER dissimilarity value between agriculture field and plant associated was 47.68% and highest value of similarity contribution which is 6.01% was by *Polypheretima elongate,* whereas the highest similarity contribution within forest and plant associated habitats was contributed by *L. terrestris* by the value of 7.45% and the average dissimilarity value was 49.24%. In wetland and plant associated the highest contribution to similarity was observed by *D bolaui* and the average dissimilarity value of 42.21% was observed between the habitats. The details of SIMPER analysis were described in the Table 4.

Table 4. Simper anlysis of earthworms across all habitats

Average dissimilarity between grassland (GL) and agricultural field (AF) (20.11%)						
Serial No.	**Species**	**Avg. abundance (GL)**	**Avg. abundance (AF)**	**Avg.dissimilarity**	**Similarity Contribution %**	**Cum.%**
1	*Amynthas spp.*	3.61	0	2.03	10.07	10.07
2	*Eutyphoeus incommodus*	0	3.16	1.78	8.83	18.9
3	*Amynthas diffringens*	0	3	1.69	8.38	27.28
4	*Millsonia anomala*	3	0	1.69	8.38	35.66
5	*Aporrectodea caliginosa*	0	2.83	1.59	7.9	43.56
Average dissimilarity between grassland (GL) and forest (F) (40.38%)						
Serial No.	**Species**	**Avg. abundance (GL)**	**Avg. abundance (F)**	**Avg.dissimilarity**	**Similarity Contribution %**	**Cum.%**
1	*Dichogaster saliens*	3.32	0	2.26	5.6	5.6
2	*Eutyphoeus waltoni*	3.32	0	2.26	5.6	11.21
3	*Octolasion lacteum*	3.32	0	2.26	5.6	16.81
4	*Lennogaster pusillus*	3.16	0	2.16	5.34	22.16
5	*Eutyphoeus incommodus*	0	3	2.05	5.07	27.22
Average dissimilarity between agricultural field (AF) and forest (F) (30.04%)						
Serial No.	**Species**	**Avg. abundance (AF)**	**Avg. abundance (F)**	**Avg.dissimilarity**	**Similarity Contribution %**	**Cum.%**
1	*Lennogaster pusillus*	3.46	0	2.31	7.69	7.69
2	*Drawida willsi*	3	0	2	6.66	14.35
3	*Dichogaster saliens*	2.83	0	1.89	6.28	20.62
4	*Metaphire posthuma*	2.83	0	1.89	6.28	26.9
5	*Eutyphoeus waltoni*	2.65	0	1.76	5.87	32.77
Average dissimilarity between agriculture field (AF) and wetland (WL) (51.11%)						
Serial No.	**Species**	**Avg. abundance (AF)**	**Avg. abundance (WL)**	**Avg.dissimilarity**	**Similarity Contribution %**	**Cum.%**
1	*Polypheretima elongata*	3.61	0	2.87	5.61	5.61
2	*Eudrilus eugeniae*	3.16	0	2.51	4.92	10.53
3	*Eutyphoeus nicholsoni*	3.16	0	2.51	4.92	15.45
4	*Amynthas diffringens*	3	0	2.39	4.67	20.12
5	*Drawida willsi*	3	0	2.39	4.67	24.79
Average dissimilarity between forest (F) and wetland (WL) (40.74%)						

Serial No.	Species	Avg. abundance (F)	Avg. abundance (WL)	Avg.dissimilarity	Similarity Contribution %	Cum.%
1	*Polypheretima elongata*	3.16	0	3.35	8.23	8.23
2	*Octochaetona surensis*	2.45	0	2.6	6.38	14.61
3	*Pheretima alexandri*	2.45	0	2.6	6.38	20.98
4	*Dichogaster bolaui*	2.24	0	2.37	5.82	26.8
5	*Eutyphoeus nicholsoni*	2.24	0	2.37	5.82	32.62

Average dissimilarity between grassland (GL) and Plant associated (PA) (50.54%)

Serial No.	Species	Avg. abundance (GL)	Avg. abundance (PA)	Avg.dissimilarity	Similarity Contribution %	Cum.%
1	*Amynthas spp.*	3.61	0	2.94	5.82	5.82
2	*Eutyphoeus nicholsoni*	3.46	0	2.83	5.6	11.42
3	*Dichogaster saliens*	3.32	0	2.71	5.36	16.78
4	*Octolasion lacteum*	3.32	0	2.71	5.36	22.14
5	*Dendrobaena octaedra*	3.16	0	2.58	5.11	27.24

Average dissimilarity between agricultural field (AF) and plant associated (PA) (47.68%)

Serial No.	Species	Avg. abundance (AF)	Avg. abundance (PA)	Avg.dissimilarity	Similarity Contribution %	Cum.%
1	*Polypheretima elongata*	3.61	0	2.86	6.01	6.01
2	*Eutyphoeus incommodus*	3.16	0	2.51	5.27	11.27
3	*Eutyphoeus nicholsoni*	3.16	0	2.51	5.27	16.54
4	*Amynthas diffringens*	3	0	2.38	5	21.54
5	*Octochaetona serrate*	3	0	2.38	5	26.53

Average dissimilarity between forest (F) and Plant associated (PA) (49.24%)

Serial No.	Species	Avg. abundance (F)	Avg. abundance (PA)	Avg.dissimilarity	Similarity Contribution %	Cum.%
1	*Lumbricus terrestris*	3.46	0	3.67	7.45	7.45
2	*Polypheretima elongata*	3.16	0	3.35	6.8	14.24
3	*Eutyphoeus incommodus*	3	0	3.18	6.45	20.69
4	*Dendrobaena octaedra*	2.83	0	2.99	6.08	26.77
5	*Lennogaster pusillus*	0	2.45	2.59	5.27	32.04

Average dissimilarity between wetland (WL) and plant associated (PA) (42.21%)

Serial No.	Species	Avg. abundance (WL)	Avg. abundance (PA)	Avg.dissimilarity	Similarity Contribution %	Cum.%
1	*Dichogaster bolaui*	0	2.65	3.77	8.93	8.93

2	*Aporrectodea caliginosa*	2.24	0	3.18	7.54	16.47
3	*Dendrobaena octaedra*	2.24	0	3.18	7.54	24.01
4	*Eutyphoeus incommodus*	2.24	0	3.18	7.54	31.55
5	*umbricus rubellus*	0	2.24	3.18	7.54	39.1
Average dissimilarity between grassland (GL) and wetland (WL) (52.86)						
Serial No.	**Species**	**Avg. abundance (GL)**	**Avg. abundance (WL)**	**Avg.dissimilarity**	**Similarity Contribution %**	**Cum.%**
1	*Eutyphoeus nicholsoni*	3.46	0	2.83	5.36	5.36
2	*Dichogaster saliens*	3.32	0	2.71	5.13	10.49
3	*Octolasion lacteum*	3.32	0	2.71	5.13	15.62
4	*Polypheretima elongata*	3.16	0	2.59	4.89	20.51
5	*Eutyphoeus sp.*	3	0	2.45	4.64	25.15

The IndVal index produced a list of indicator species for each group of sites: *Millsonia anomala* (p = 0.005; statistical value: 0.751) was a greater indicator of the grassland, whereas *Allolobophora parva* sp. was strongly correlated (p = 0.005; statistical value: 0.950) with the agriculture. *Lumbricus terrestris* (p = 0.005; statistical value: 1.000) was significantly associated with the forest. The conditional probability or positive predictive value of the species and the conditional probability of finding the species at sites and those species with the highest IndVal value for the set of all the samples from each habitat (e.g. *Amynthas spp., Octolasion lacteum, Dichogaster saliens, Polypheretima elongate)*. However, the species like *Eisenia fetida, Aporrectodea rosea, Drawida calebi, Lampito mauritii, Lumbricus terrestris, Octochaetona beatrix* were not amenable to statistical testing because of the lack of an external group for comparison.

The five habitats differed significantly in the diversity indices, with grassland having the highest species richness (S) with 29 species, and the wetland recorded the lowest richness with 15 species. The highest abundance (N) was also observed in the grassland (258), and the lowest in the wetland (75). Density (d) refers to the number of individuals per unit area, where grassland has a density of 0.03921, which is relatively low compared to wetland with 0.0752. Evenness (J') measures how evenly the individuals are distributed across the species. Values close to 1 indicate even distribution, and values closer to 0 indicate more dominance by a few species. All habitats have high evenness, ranging from 0.9608 (grassland) to 0.9248 (wetland). Shannon-Weiner Index (H'): The diversity index considers both species richness and evenness, with grassland observed to have the highest diversity with an H' value of 3.301, and the wetland has the lowest with 2.647. Similarly, Simpson's Index of Diversity (1 – Lambda) estimates the probability that two individuals randomly selected from the sample belong to different species. Higher values indicate higher diversity, where grassland and agricultural fields observed the highest values, 0.9608 and 0.9622, respectively, indicating more diversity (Table 5).

Table 5. Average values of diversity indices in each habitat

Habitats	**Diversity indices**					
	S	**N**	**d**	**J'**	**H'(loge)**	**1-Lambda'**
Grassland	29	258	0.03921	0.9805	3.301	0.9608
Agricultural field	32	255	0.03782	0.9733	3.373	0.9622
Forest	24	137	0.04854	0.9761	3.102	0.9515
Wetland	15	75	0.0752	0.9774	2.647	0.9248
Plant associated	16	72	0.07446	0.9683	2.685	0.9255

Functional traits analysis

In this study, five morphometric traits were selected (ecological category, body length, setae shape, development of anterior musculature, and body color) and their association with ecological function in earthworms was investigated. Based on the three ecological categories, it was revealed that epigeic earthworms were typically uniformly pigmented, had medium body length, poorly developed anterior musculature, and straight setae. In contrast, anaeic earthworms were characterized by a dorsally pigmented body, smaller body length, well-developed anterior musculature, and curved setae. Edogeic earthworms, on the other hand, were non-pigmented, had large body size, well developed anterior musculature and well-developed straight setae. These results suggest that morphometric traits can provide valuable information about the ecological function of earthworms.

Our analysis of earthworms in the agricultural field habitat revealed that the dominant traits were epigeic, large body length, not visible setae, poorly developed anterior musculature, and uniformly pigmented body. The epigeic earthworms were closely associated with traits such as uniformly pigmented, not visible setae and weak anterior musculature (AM). In contrast, anaeic earthworms were characterized by dorsally pigmented body and curved setae (Fig. 5). Similarly, endogeic earthworms were identified as non-pigmented, with straight setae and well-developed anterior musculature (Fig. 5). These observations suggest that morphometric traits can provide valuable insights into the characteristics of earthworms in different habitats. Similarly, in grassland habitats, anecic and endogeic earthworms were found to be dominant, with moderate body length, non-developed or well-developed anterior musculature (AM), not visible or curved setae, and dorsally pigmented or non-pigmented bodies. Endogeic worms were characterized by non-pigmented bodies, straight setae, and welldeveloped anterior musculature, while anecic species were characterized by dorsally pigmented bodies and curved setae. The traits of uniformly pigmented, not visible setae, and weak anterior musculature closely resembled those of epigeic earthworms (Fig. 6).

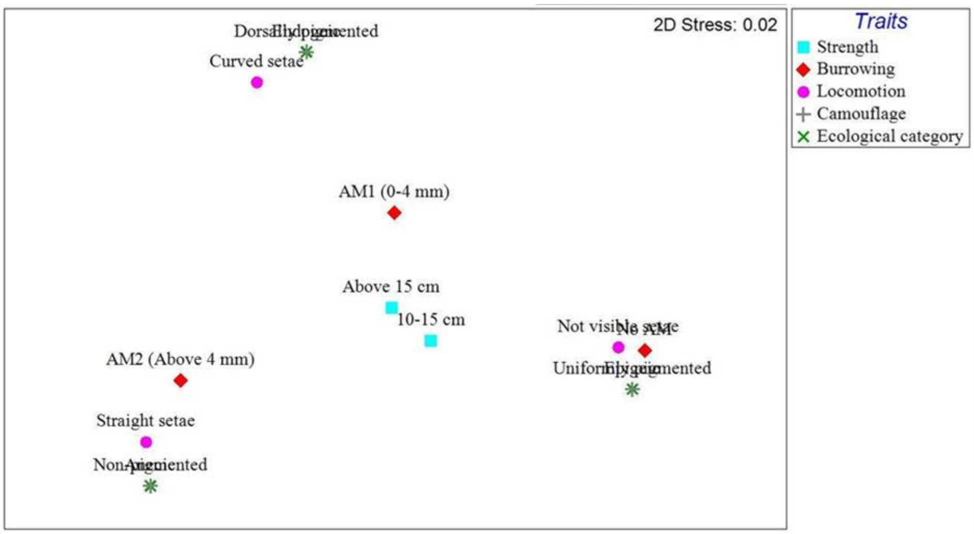


Figure 5: Traits distribution pattern in agricultural field

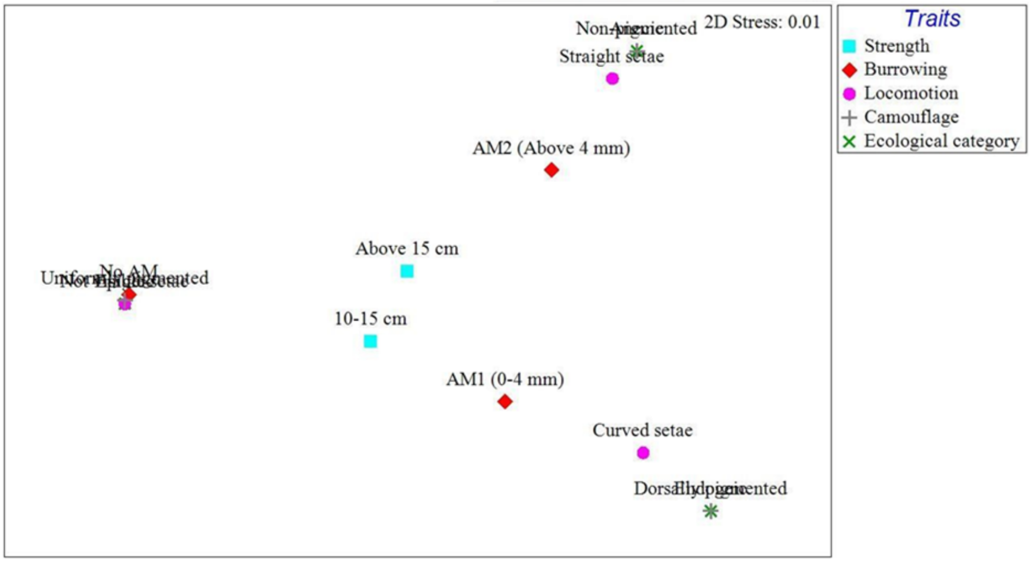


Figure 6: Traits distribution pattern in grassland habitat

Forest habitat was not dominated by any specific trait; however, endogeic traits were found in lower numbers, while the remaining traits were found in moderate numbers. According to the Bray-curtis nMDS similarity index (Fig.7), trait attributes like dorsally pigmented body, curved setae, and developed AM were associated with anecic species, while trait attributes like uniformly pigmented body, not visible setae, and weak AM were associated with epigeic species. Endogeic species shared a well-developed AM, straight setae, and a non-pigmented body. The distribution pattern of traits is shown in Fig. 7.

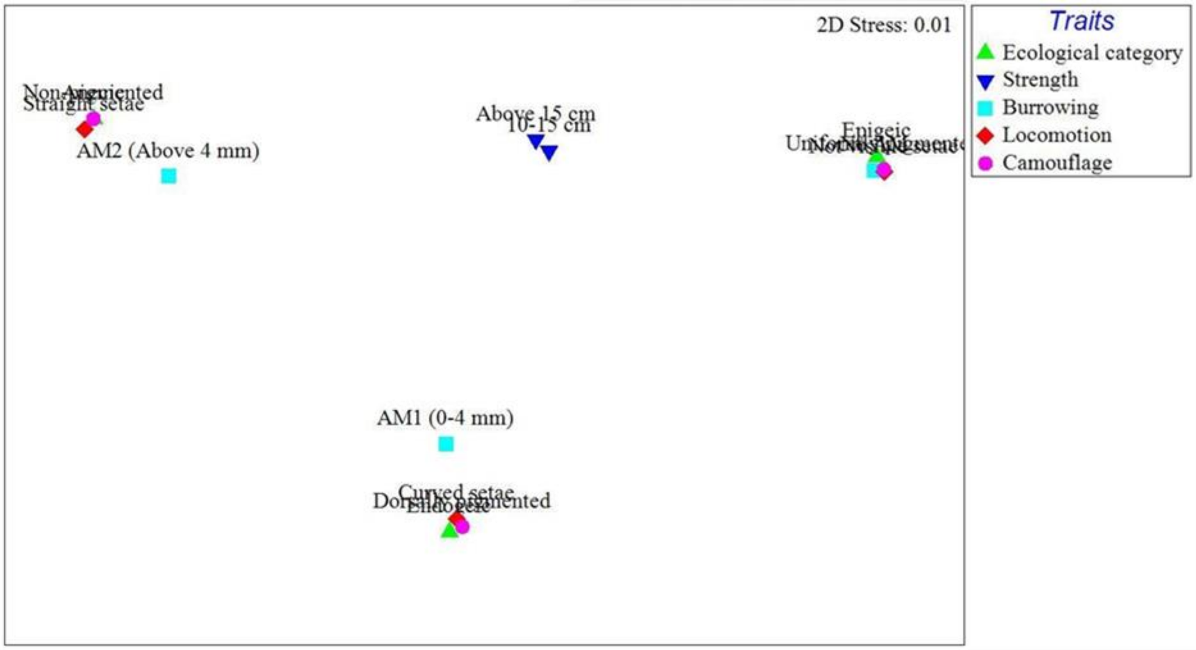


Figure 7: Traits distribution pattern in forest ecosystem

In wetland ecosystem the endogeic trait categories earthworms were absent and the remaining traits were found to be in lower numbers. The distribution pattern of traits revealed that epigeic species were associated with uniformly pigmented, poorly developed anterior musculature (AM), and non-visible setae, anecic species with dorsally pigmented body, moderately developed anterior musculature (AM) and curved setae, whereas endogeic species were attributed by non-pigmented body and strong straight setae. Figure 8 depicts the pattern of trait distribution. We discovered that species with endogeic traits were absent from plant associated sites, and the remaining ecological traits were in short supply. However, the distribution pattern revealed that uniformly pigmented and not visible setae were closely related to epigeic worms, whereas dorsally pigmented, curved setae, and moderately developed AM were closely related to anecic species. Endogeic earthworms were associated with non-pigmented bodies and straight setae, as with other habitats (Fig. 9)

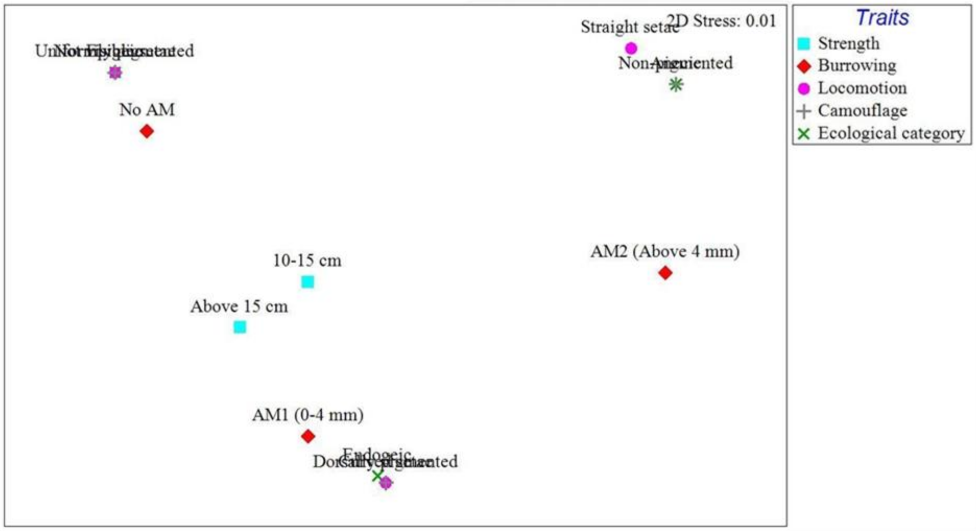


Figure 8: Traits distribution pattern in wetland ecosystem

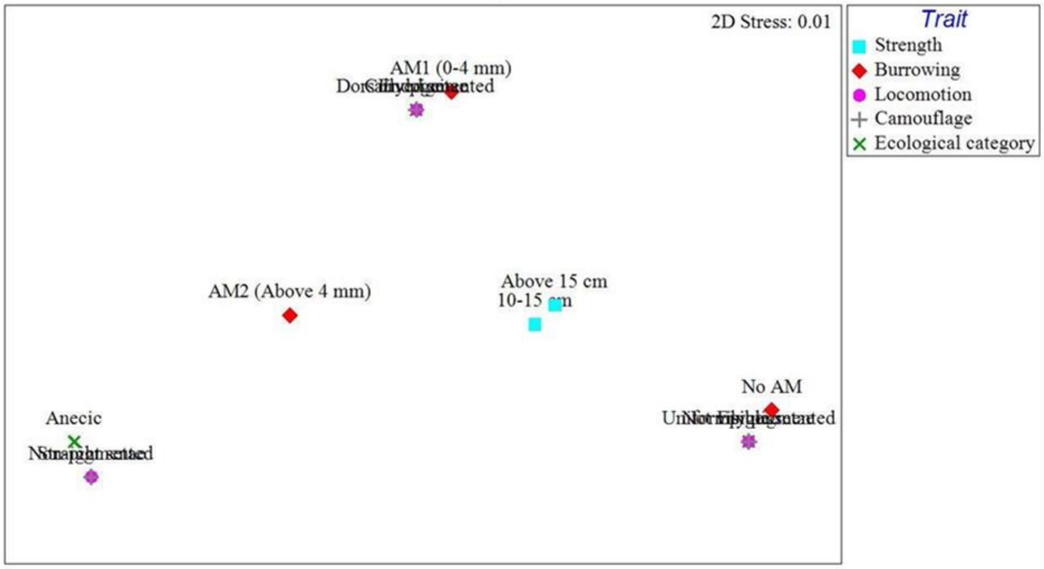


Figure 9: Traits distribution pattern in plant associated habitat

The schematic model represents the exact trend of each functional trait of each habitat. The relative abundance of each trait was plotted as an area graph, and a schematic figure was prepared to show the pattern according to the habitats. For example, the agriculture field and grassland habitats favour the dominance of all ecological traits whereas forest, wetland and plant associated ecosystems were not favouring the dominance of any trait (Fig. 10).

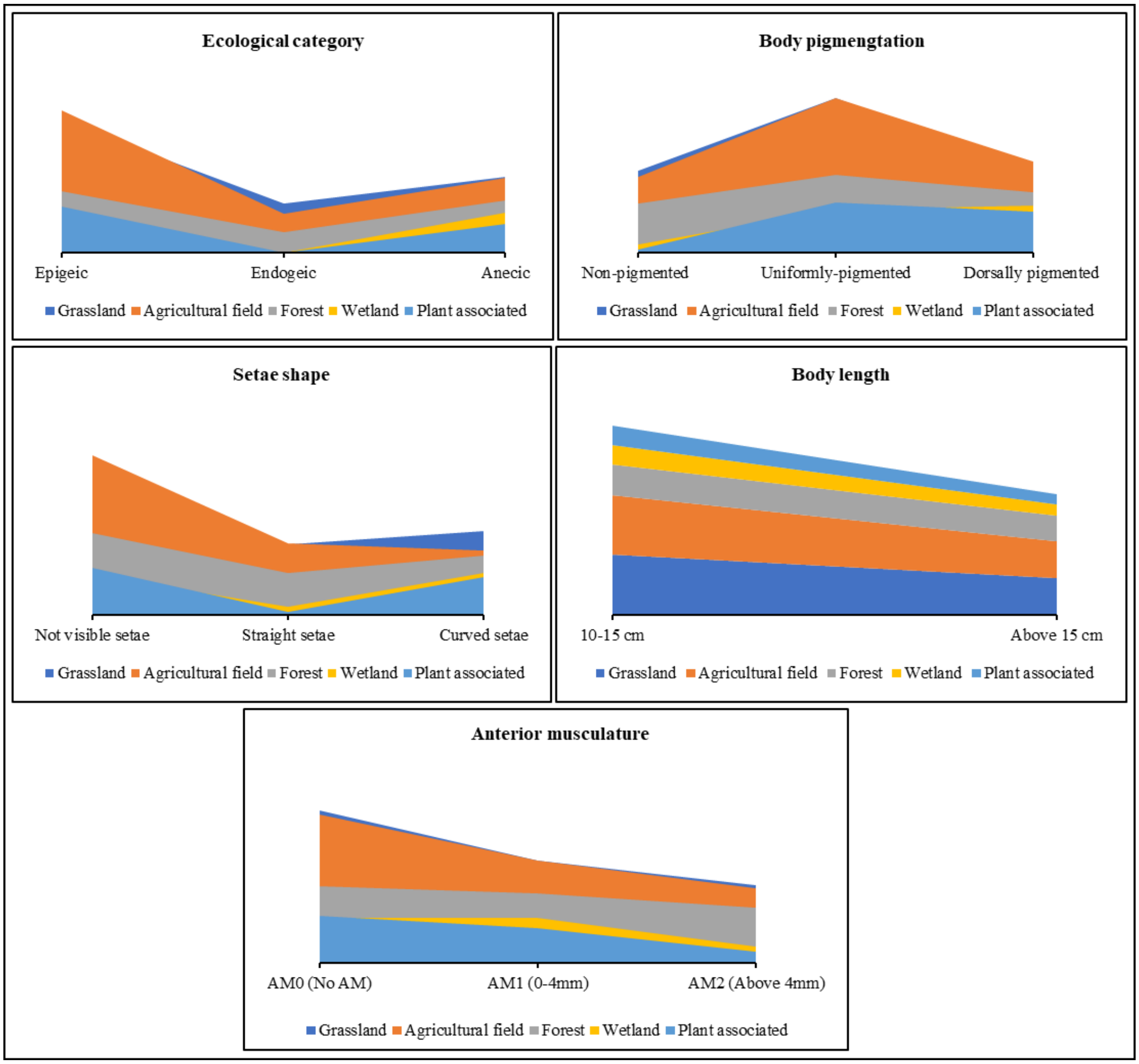
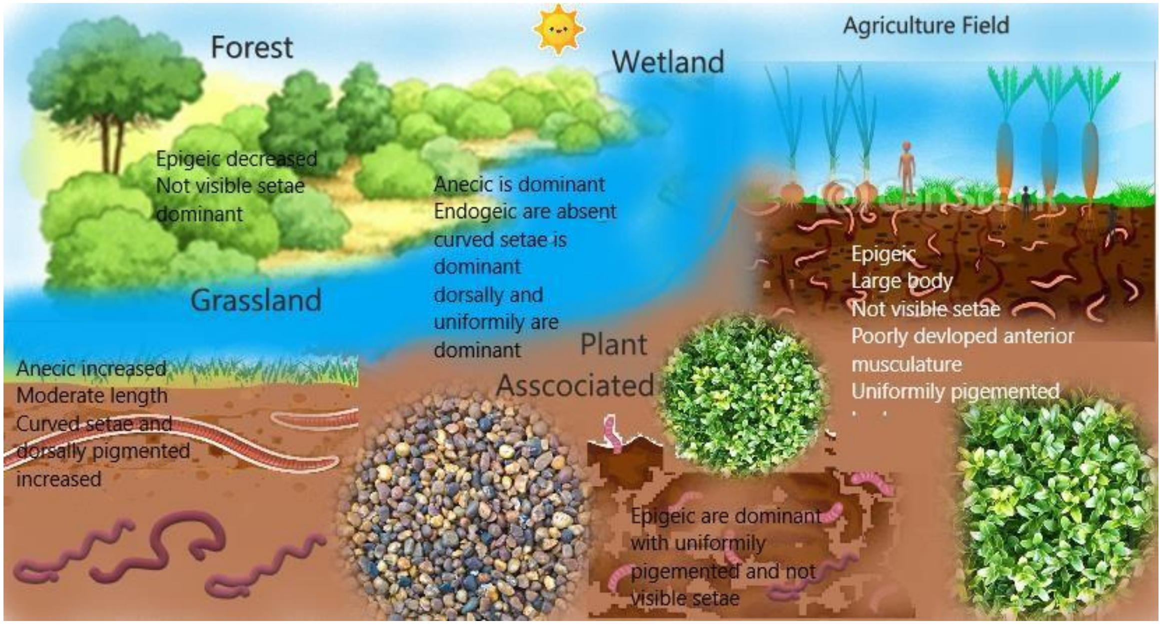


Figure 10: Schematic model illustrating the functional traits distribution pattern of earthworms in each habitat



Graphical abstract

Discussion

The highest and lowest abundance and diversity of earthworms were observed in grassland site and plant associated habitat site respectively. The earthworm abundance in all the habitats vary in number ranging from only few individuals to more abundant, which depends on the physicochemical characteristic of the soil and the climatic condition of that habitat (Kale & Karmegam, 2010; Lee, 1985). The results of the DistLM analysis indicate that soil parameters play a significant role in determining species abundance in various environments. In forest and wetland habitats, the presence of sand and bulk density, respectively, have the greatest impact. In a study, Yvan et al., (2012) observed that soil texture influences the activity and growth of earthworm (Yvan et al., 2012). In the current study we observed higher clay content favors the growth and abundance of earthworm which similarly reported in the study of Singh et. al., (2021) (Singh et al., 2021). Meanwhile, available N, clay, and organic carbon are important factors in plant-associated habitats. Agricultural fields and grasslands, on the other hand, show little effect from soil parameters on species abundance. Soil properties such as texture and pH can vary significantly across habitats, with agriculture fields having the highest sand and clay content and wetland having the highest silt content. Higher clay content and slightly alkaline pH (near 8.07) were found to promote earthworm growth and abundance in this study, similar to findings by other researchers. Most studies show that earthworms can thrive in a pH range of 5.0 to 8.0, with neutral pH promoting the greatest abundance (De Wandeler et al., 2016).

The levels of organic carbon and available N play a crucial role in determining earthworm abundance and diversity across habitats, as highlighted by several studies in the literature (Xie et al., 2022). Our study found that the highest levels of organic carbon and available N were present in agricultural fields and were associated with increased earthworm abundance and diversity (Bartz et al., 2013; Jänsch et al., 2013). This observation is consistent with the findings of several studies that have reported a positive relationship between soil organic carbon content and earthworm populations (Bartz et al., 2013; Jänsch et al., 2013) However, the relationship between organic carbon levels and earthworm populations is not always straight forward (Lavelle & Spain, 2001). In our study, we also found that plant-associated soil had high levels of organic carbon, but earthworm abundance and diversity were low. This finding is in line with the results of other studies, which have shown that earthworm populations are influenced by a range of factors, including soil structure, pH, and nutrient availability (De Wandeler et al., 2016). Moderate to high rainfall, temperature and humidity promote the earthworm abundance that might be favoring their metabolic and reproductive rate.

Similarly, our study found that earthworm populations were abundant and diverse in grasslands, despite low organic carbon levels. This observation supports the idea that earthworms can thrive in a range of conditions, as long as other essential factors, such as soil structure and nutrient availability, are favorable(De Wandeler et al., 2016). The results indicate that soil parameters and earthworm species play a crucial role in shaping earthworm populations in different habitats. The specific patterns of earthworm diversity and abundance varied among habitats, suggesting that soil parameters and earthworm species interact differently in different habitats. Overall, all habitats were found to be significantly different from each other, emphasizing the importance of considering the unique characteristics of each habitat when studying earthworm populations.

The findings of the study provide insight into the distribution of earthworm species and their abundance across different habitats. The study showed that grassland habitats had the highest levels of earthworm diversity and abundance followed by agricultural fields, forests, wetlands, and plant-associated habitats. This highlights the important role that different habitats play in shaping earthworm populations. The significant observation was the dominance of *Eisenia fetida* as the most common species across all habitats. Hussain et al. (2022) also reported similar findings, suggesting the resilience and adaptability of *E. fetida* in different climatic conditions and its high reproductive rate as key factors contributing to its dominance (Hussain et al., 2022).

Additionally, the study found that certain species, such as *Millsonia anomala*, were only present in specific habitats, whereas, *Dichogaster saliens*, *Metaphire posthuma, Octolasion lacteum* and *Pellogaster bengalensis*, were found in multiple habitats. This species-specific distribution was previously reported in studies of earthworm species compositions in various grassland, agricultural, and forest soils (Satchell, 1983).

Apart from taxonomic information studying morphological traits of earthworms helps us to understand the adaptation of earthworms to different habitats and to know the specific function of earthworms in each habitat (Blakemore, 2000). This can lead to a better understanding of the role of earthworms in maintaining soil health and fertility, and their importance as indicator of soil quality (Satchell, 1983). The morphological traits of earthworms, such as body length, setae shape, development of anterior musculature (AM), and body color, are directly related to their ability to perform specific functions, such as burrowing and feeding within the soil ecosystem. By understanding the correlation between morphological traits and ecological function, researchers can better understand the role that earthworms play in maintaining soil health and fertility.

The morphological traits of earthworms are important to study because they are related to their ecological function. It has been found that different ecological categories of earthworms, such as epigeic, anecic, and edogeic, were characterized by different morphological traits, such as body pigmentation, body length, development of anterior musculature, and setae shape. These morphological traits are associated with different functions such as protection from predators, burrowing, and camouflage etc (Hsu et al., 2023).

For example, epigeic earthworms were uniformly pigmented and had medium body length, poorly developed anterior musculature, and not visible setae, which were adaptations for their surface-dwelling lifestyle. Anecic earthworms were dorsally pigmented, had smaller body length, well-developed anterior musculature, and curved setae, which were adaptations for their burrowing lifestyle. Edogeic earthworms were non-pigmented, had large body size, well-developed anterior musculature, and straight setae, which adapted for the deep soil burrowing lifestyle. The study was conducted by consulting the previous studies of Bouche (1977) who investigated the influence of body size on the burrowing activity of earthworm (Bouché, 1977) and Marichal et al. (2017) who investigated the impact of morphological traits on the burrowing and foraging behaviors (Marichal et al., 2017). Some other studies conducted by Satchell (1983), and Julka and Senapati (1987), explored the relationship between earthworm species composition and habitat type. These studies provided a foundation for understanding the role of morphological traits in earthworm ecology and highlighted the need of further research in this area. the results of this study provide important insights into the relationship between morphological traits and ecological function in earthworms and contribute to our understanding of earthworm diversity and distribution in different habitats. Further research is needed to fully understand the mechanisms driving the evolution of these traits and their impacts on earthworm populations and ecosystem functioning (Satchell, 1983;Julka & Senapati, 1987).

In the present study, it was observed that the morphometric traits of earthworms vary depending on their habitat. Previously numerous studies were conducted to understand the the relationship between earthworm morphology and habitat (Edwards et al., 1995)(Marichal et al., 2017). In the case of agricultural fields, earthworms were found with inclined traits such as epigeic, uniformly pigmented bodies, not visible setae, and weak anterior musculature. The presence of these traits supports the presence of high organic content in the upper soil surface. This is because of the frequent tillage practices that increase the organic matter inputs into the soil (Edwards et al., 1995). In contrast, grasslands favor anecic and endogeic earthworms, which display a range of traits, including moderate body length to large body length, non-developed to well-developed anterior musculature, and dorsally pigmented to non-pigmented bodies (Edwards et al., 1995). This dominance of anecic and endogeic earthworms in grasslands might be due to the low risk of predators and disturbance from anthropogenic activities, compared to agricultural fields. Forests were observed with no dominance of any specific trait, whereas wetland habitats lack endogeic species due to anoxic conditions created by deep ground water (Edwards et al., 1995).

Additionally, the distribution of morphometric traits varied depending on the habitat, with endogeic earthworms being absent from plant-associated sites. Epigeic earthworms were closely related to uniformly pigmented bodies, not visible setae, and weak anterior musculature, while anecic earthworms were closely related to dorsally pigmented bodies, curved setae, and moderately developed anterior musculature. Endogeic earthworms were closely related to non-pigmented bodies and straight setae (Marichal et al., 2017). Further, this study highlights the importance of morphometric traits in determining the ecological function of earthworms and the inclination towards their habitat.

Conclusion

This study provides a comparative and comprehensive understanding of the distribution pattern of earthworms and their morphometric traits across different terrestrial ecosystems. The study revealed that grassland habitats had the highest levels of earthworm diversity and abundance, followed by agricultural fields, forests, wetlands, and plant-associated habitats. The earthworm community structure and functionality are influenced by diverse habitat gradients, with species composition and abundance varying significantly across different habitat types, indicating environmental responsiveness. The study emphasizes the role of soil parameters like texture, pH, organic carbon, and nitrogen in determining earthworm populations. It observes *Eisenia fetida* as the most common species and highlights the species-specific distribution of earthworms. It also highlights the importance of understanding earthworm morphological traits and their adaptation to different environmental conditions.

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