



Effects of millipede activity on soil nutrient dynamics under controlled microcosm conditions

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Abstract

Millipedes are important soil detritivores that contribute significantly to litter decomposition and nutrient cycling in terrestrial ecosystems. However, experimental evidence on the species-specific effects of millipedes on soil nutrient dynamics remains limited. This study investigated the effects of three millipede species—*Aulacobolus graveyi*, *Anoploidesmus saussurii*, and *Phyllogonostreptus* sp.—on selected soil properties using a laboratory microcosm experiment. Microcosms containing soil and leaf litter were maintained for 60 days with and without millipedes. At the end of the experiment, soil pH, electrical conductivity (EC), soil organic carbon (SOC), nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were analysed.

All measured soil parameters differed significantly among groups (ANOVA: $F = 15.41\text{--}357.24$, $P < 0.05\text{--}0.001$, $R^2 = 0.85\text{--}0.99$). Compared with the control, soil containing millipedes showed significantly higher pH, SOC, N, P, K, Ca, Mg, and EC values (Tukey's HSD, $p < 0.05$). *A. graveyi* recorded the highest SOC content (3.95 ± 0.03). Phosphorus levels were highest in *A. graveyi* and *Phyllogonostreptus* sp. (176.52 ± 1.12 and 177.42 ± 1.84 , respectively), and both differed significantly from *A. saussurii* (140.12 ± 5.53). In contrast, N, K, Ca, Mg and EC did not differ significantly among the three millipede species. In the present study, most significant differences occurred between the control and millipede-treated soils. Millipede activity significantly improved nutrient availability under controlled conditions. Species-specific differences in soil modification suggest that millipedes contribute differently to nutrient cycling and ecosystem functioning.

Keywords: *Aulacobolus graveyi*, *Anoploidesmus saussurii*, *Phyllogonostreptus* sp., microcosms, soil parameters

Introduction

Soil macroinvertebrates play an important role in decomposition, nutrient cycling, soil formation, and the maintenance of soil fertility (Snyder & Hendrix, 2008; Bardgett & Van der Putten, 2014; Angst *et al.*, 2024) ^[1, 8, 28]. Through their biological activities, millipedes alter the physical, chemical, and biological characteristics of soil, contributing to ecosystem processes.

Among soil macrofauna, millipedes (Diplopoda) are one of the most abundant groups of litter-feeding detritivores in forest ecosystems (Golovatch & Kime, 2009; Nzoko-Fiemapong *et al.*, 2023) ^[17, 29]. They feed mainly on dead plant material and play an important role in the breakdown of leaf litter. During feeding, millipedes fragment litter into smaller particles, increasing the surface area available for microbial colonization and decomposition (Cárcamo *et al.*, 2000; David, 2014) ^[5, 8]. Their fecal pellets contain partially decomposed organic matter and nutrients, which are returned to the soil and become available for further decomposition and nutrient cycling (Tajovský *et al.*, 1992) ^[23]. Through these activities, millipedes promote the decomposition of organic matter and facilitate the release of nutrients into the soil.

Previous studies have shown that millipedes can influence microbial activity, nutrient mineralization, soil aggregation, and nutrient availability (Maraun & Scheu, 1996; Kaneko, 1999; Toyota *et al.*, 2006; Fujimaki *et al.*, 2010) ^[10, 14, 24, 30]. Their feeding and burrowing activities promote the incorporation of organic matter into soil and contribute to humus formation (Baluchamy and Radhakrishnan, 2023) ^[2]. Millipedes have also been reported to influence nitrogen and phosphorus cycling, highlighting their importance in

maintaining soil health and fertility (Toyota & Kaneko, 2012; Wang *et al.*, 2018) ^[7, 26].

Despite their ecological importance, millipedes have received less attention than many other soil organisms in studies of nutrient dynamics. Most previous studies have focused on litter decomposition, microbial responses, or the effects of individual species on specific soil processes (Kaneko, 1999; Toyota *et al.*, 2006; Fujimaki *et al.*, 2010) ^[10, 14, 24]. Comparative studies examining how different millipede species influence soil chemical properties under controlled conditions remain limited. As a result, species-specific contributions of millipedes to nutrient cycling and soil fertility are still not fully understood.

Microcosm experiments provide an effective approach for studying the effects of soil fauna under controlled conditions while reducing environmental variation (Moore *et al.*, 1996; Chang *et al.*, 2024) ^[6, 16]. Such experiments allow the direct assessment of faunal influences on soil processes and nutrient dynamics. The present study addresses the limited experimental information available on the influence of millipedes on soil nutrient dynamics under controlled conditions. The findings contribute to a better understanding of the ecological role of millipedes in nutrient cycling and soil ecosystem functioning.

Materials and methods

After a pilot survey, three commonly occurring millipede species *Aulacobolus graveyi*, *Anoploidesmus saussurii*, and *Phyllogonostreptus* sp. (Fig 1), were hand-collected from shaded leaf litter habitats in Edappavoor (9°20'58" N, 76°45'34" E), in Pathanamthitta District, Kerala, India. Millipede specimens were identified based on external

morphological characters and male gonopod structures using published taxonomic descriptions and keys (Decker & Tertilt, 2012, Silvestri, 1916) [7, 20]. The study area experiences high humidity (>70%) and significant rainfall during the southwest and northeast monsoon seasons, which contribute to moist soil and abundant organic litter, conditions that favor millipede abundance and activity. After collection, the species were acclimatized under laboratory conditions for 48 h prior to experimentation. Surface soil (0–15 cm) was collected from the above site to maintain the natural microbial community. Freshly fallen leaf litter from dominant and abundant native plant species (*Mangifera indica*, *Flacourtia jangomas*, *Nephelium lappaceum*) was collected from the sampling site, cleaned, and air-dried. Litter was cut into uniform pieces (~2 cm) and oven-dried at 60 °C for 48 h to ensure moisture uniformity. The experiment was conducted under laboratory conditions at a temperature of $27 \pm 2^\circ\text{C}$ and a relative humidity of $75 \pm 5\%$. Microcosms were established using plastic containers (30 cm × 40 cm × 7 cm) filled with 1.5 kg homogenized soil and 50 g of dried leaf litter. Soil moisture was maintained by adding water when required. The containers were checked regularly to ensure adequate moisture. The experimental design included the following groups; each replicated three times.

Groups	Description
Control	Soil + leaf litter (without millipedes)
T1	Soil + leaf litter + <i>A. graveyoli</i>
T2	Soil + leaf litter + <i>A. saussurii</i>
T3	Soil + leaf litter + <i>P. sp.</i>

Microcosms were maintained under controlled conditions for 60 days. Moisture was maintained by periodic sprinkling with distilled water to keep the soil wet. No additional food was provided during the experimental period. All microcosms were established using soil collected from the same location to ensure similar initial soil conditions across all the groups.

Sampling and Analytical Procedures

At the end of the experimental period, soil samples were collected from each microcosm after removing the litter debris. Soil chemical analyses were performed on air-dried soil samples, and the results are expressed on a dry-weight basis. Composite soil samples were analyzed for the following: Soil pH and electrical conductivity (EC) were determined in a 1:2.5 (w/v) soil-to-water suspension using a pH meter and conductivity meter, respectively. Soil organic carbon (SOC) was estimated by the wet oxidation method (Walkley and Black, 1934) [27]. Available nitrogen was determined using the Kjeldahl method (Bremner and Mulvaney, 1982) [4]. Available phosphorus was estimated by Olsen's method (Olsen *et al.*, 1954) [18]. Exchangeable potassium was analyzed according to Jackson (1967) [13]. Exchangeable calcium and magnesium were determined following the procedure described by El Mahi *et al.* (1987) [9].

Statistical Analysis

The normality of the data was examined using the Shapiro–Wilk test. Treatment effects were analyzed by one-way ANOVA, followed by Tukey's HSD post hoc test for multiple pairwise comparisons whenever significant

differences were found. Statistical significance was determined at $p < 0.05$, $p < 0.01$, and $p < 0.001$. Principal Component Analysis was used to examine the overall pattern of variation in soil properties among the microcosms. It will also help identify the soil parameters that contributed most to the differences. Statistical analyses were performed using OriginPro 2023. [32]

Results and discussion

This study showed that millipede activity altered several soil properties under controlled conditions. Soils containing millipedes generally had higher nutrient levels than the control, indicating their role in nutrient release during litter decomposition. The three species did not affect all soil parameters in the same way, suggesting differences in their influence on soil processes. These findings support the view that millipedes contribute to nutrient turnover and help maintain soil quality in terrestrial ecosystems

Soil nutrient analysis

Mean values of the soil chemical properties differed among the four groups (Control, *A. graveyoli*, *A. saussurii*, and *Phyllogonostreptus sp.* (Fig 1). The Shapiro–Wilk test showed that all data were normally distributed ($p > 0.05$). Therefore, the data were analysed using one-way ANOVA followed by Tukey's HSD test.

One-way ANOVA showed significant differences among treatments for all soil properties: pH, $F(3, 8) = 32.17$, $p < 0.001$; SOC, $F(3, 8) = 39.47$, $p < 0.001$; nitrogen, $F(3, 8) = 15.41$, $p < 0.01$; phosphorus, $F(3, 8) = 357.24$, $p < 0.001$; potassium, $F(3, 8) = 49.85$, $p < 0.001$; calcium, $F(3, 8) = 17.58$, $p < 0.001$; magnesium, $F(3, 8) = 16.27$, $p < 0.05$; and electrical conductivity, $F(3, 8) = 15.88$, $p < 0.01$. Tukey's HSD test showed that SOC and phosphorus differed significantly among the millipede species, while pH, nitrogen, potassium, calcium, magnesium and electrical conductivity did not differ significantly among the three millipede treatments. The superscript letters in Fig 2 describes the results of the Tukey's HSD test.

Compared with the control, soils containing millipedes had higher nutrient levels and greater organic carbon content. These changes may be due to leaf litter breakdown, feeding activity, and the production of fecal pellets, which help speed up decomposition and nutrient release. Similar effects of millipedes on litter breakdown and nutrient cycling have been reported by Cárcamo *et al.* (2000) [5]. The higher pH and electrical conductivity in the millipede treatments may be due to the release of soluble ions and base cations during decomposition (Smit and Van Aarde, 2001) [21]. The differences observed in SOC and phosphorus among the millipede species may be related to differences in feeding behavior and litter processing. Wang *et al.* (2018) [26] reported that millipedes can increase the availability of phosphorus in soil, while Cárcamo *et al.* (2000) [5] suggested that enhanced microbial activity may promote phosphorus cycling. Calcium, magnesium, and nitrogen also differed significantly among control group and microcosm with millipede. The process of litter breakdown by millipedes promotes bacterial growth, contributing to elevated nitrogen content in feces and enhanced microbial activity throughout the soil system (Rannavre & Donde, 2023) [31].

Species-specific effects of millipedes

All three millipede species increased nutrient levels relative to the control group. However, their effects were not the same for all soil properties. ANOVA and Tukey's HSD test were used to compare the treatment means. The test showed that *A. gravelyi* had significantly higher SOC content (3.95 ± 0.03), than *A. saussurii* (2.40 ± 0.38) and *Phyllogonostreptus* sp. (2.42 ± 0.39). Tukey's HSD test indicated that phosphorus levels were significantly higher in the *A. gravelyi* (176.52 ± 1.12) and *Phyllogonostreptus* sp. (177.42 ± 1.84) treatments than in the *A. saussurii* treatment (140.12 ± 5.53), and all three millipede treatments had significantly higher phosphorus levels than the control (108.94 ± 0.96) (Fig 1). In contrast, no significant differences were observed among the three millipede species for pH, nitrogen, potassium, calcium, magnesium and electrical conductivity, although these values were significantly higher than the control.

The differences in SOC and phosphorus may be due to differences in the feeding behavior of the millipede species. Iwashima *et al.* (2010)^[12] reported that different millipede species consume different amounts of leaf litter and soil, which can influence nutrient release. Although this was not examined in the present study, it may explain the higher SOC in *A. gravelyi* and the higher phosphorus levels in *A. gravelyi* and *Phyllogonostreptus* sp. Smit and Van Aarde

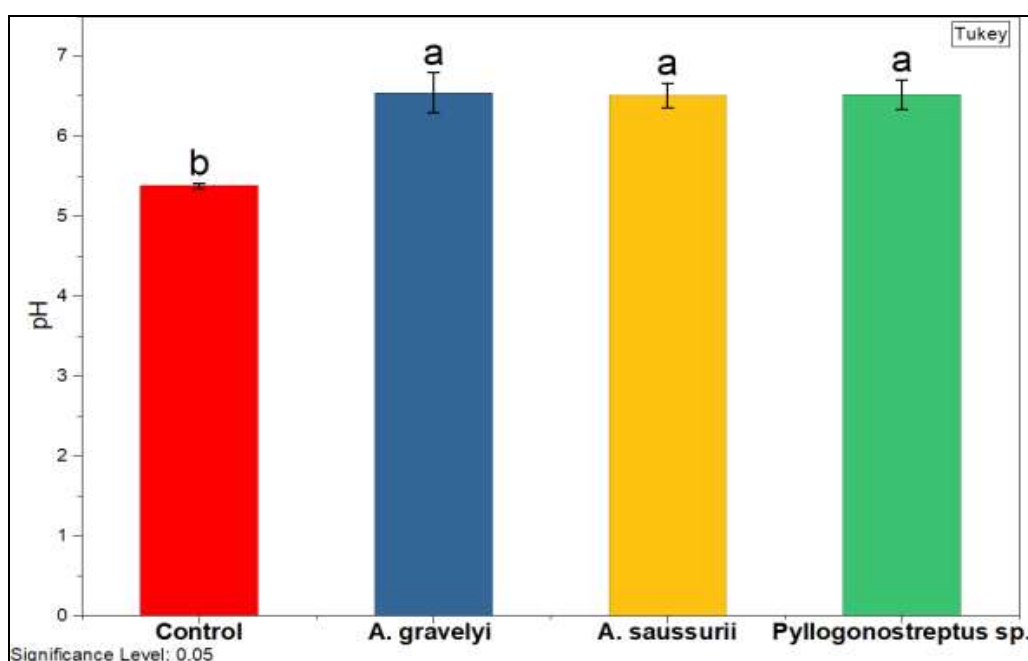
(2001)^[33] also reported that different millipede species vary in their contribution to soil nutrient enrichment. These findings suggest that the effect of millipedes on soil nutrients depends not only on their presence but also on the species.

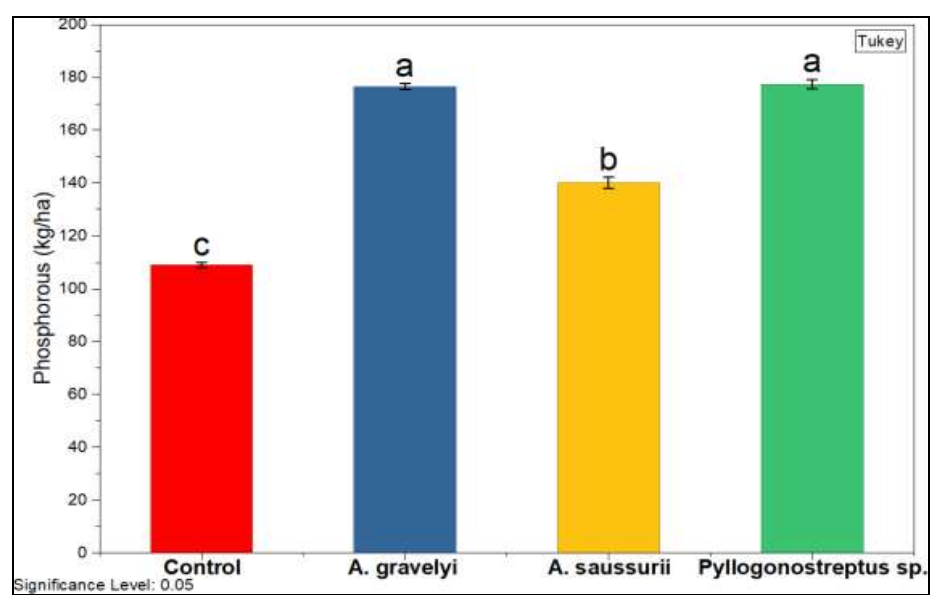
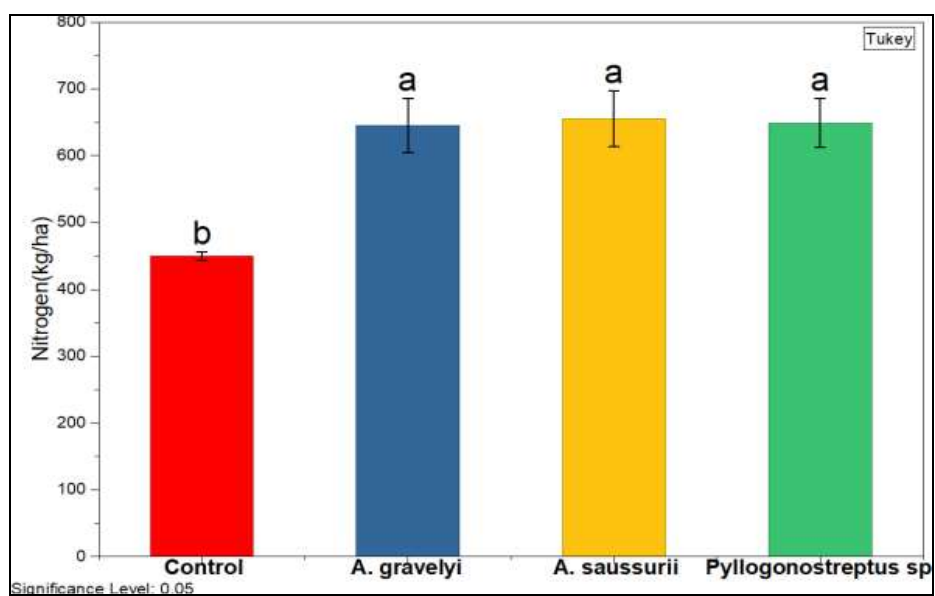
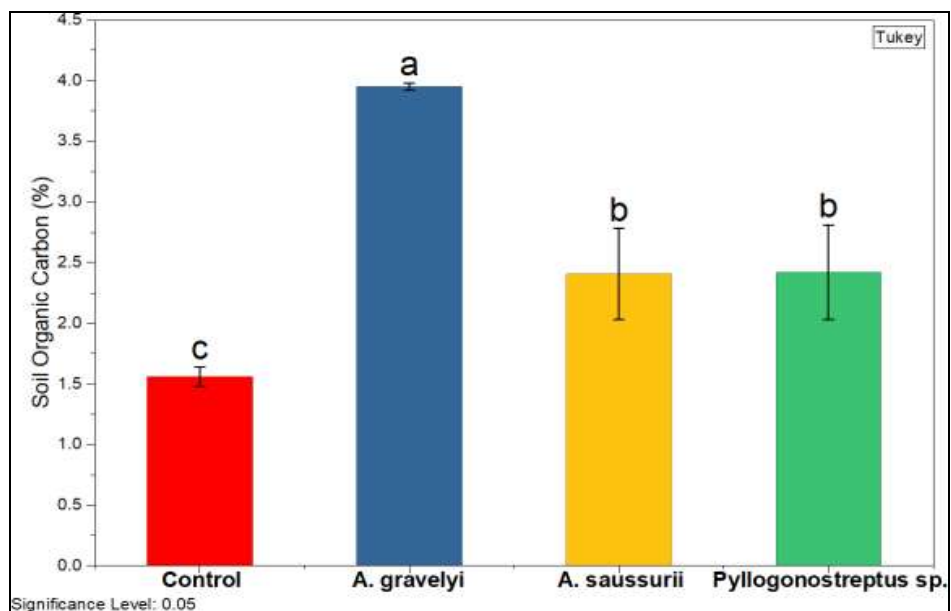
Principal Component Analysis (PCA)

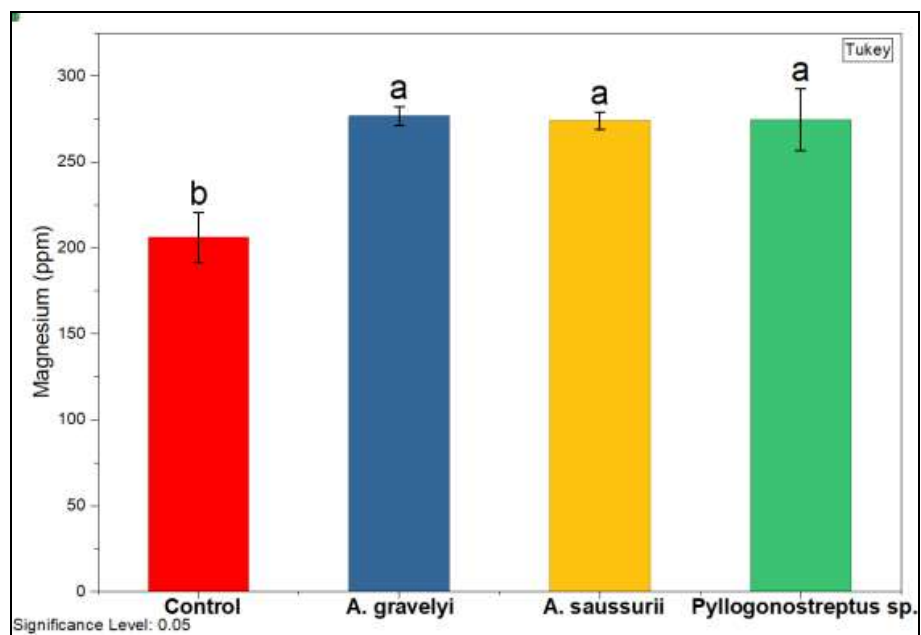
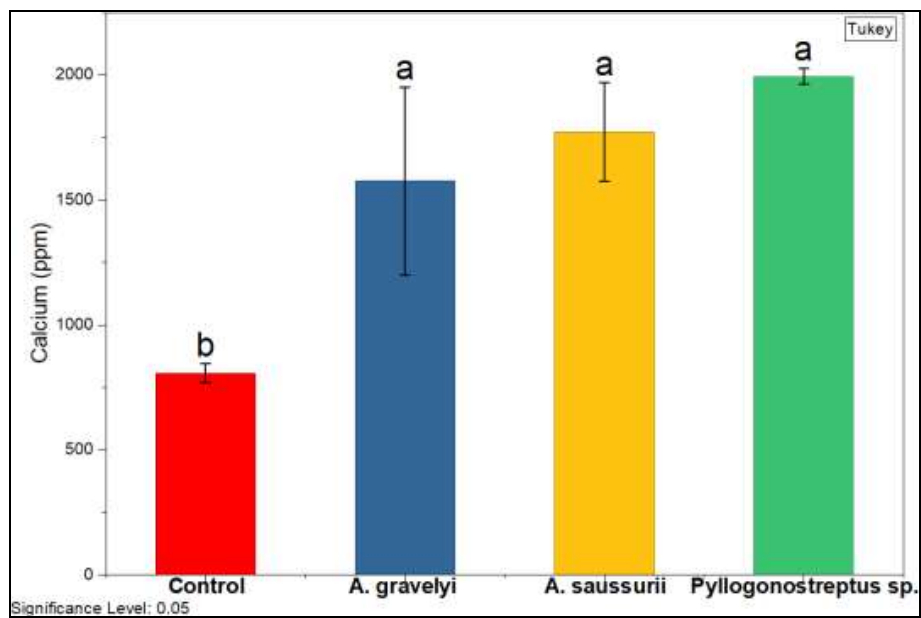
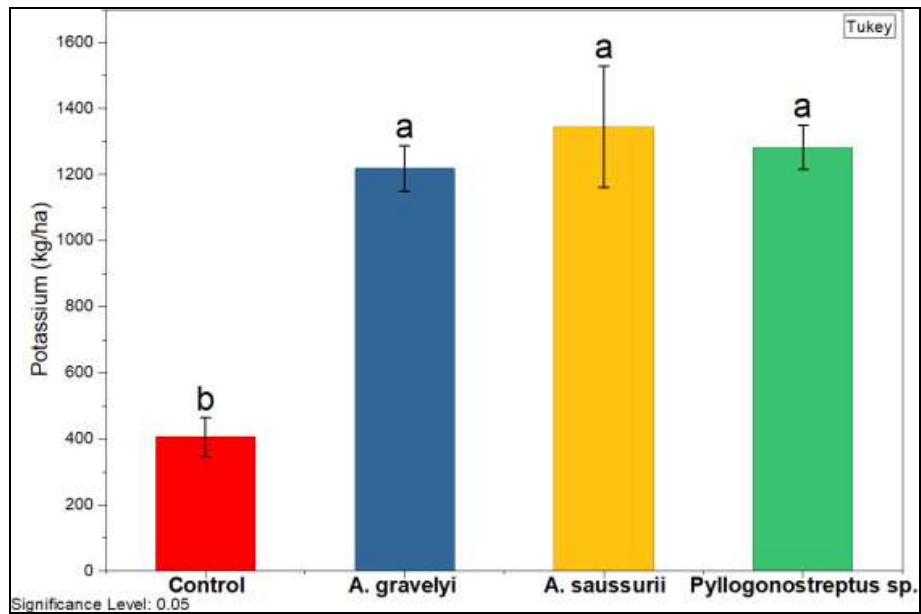
Principal Component Analysis (PCA) supported the univariate statistical results by showing clear separation between control and millipede-treated microcosms in multivariate space. PC1 explained 81.40% (eigenvalue = 6.547) of the total variance, whereas PC2 explained 8.99%, together accounting for 90.87% of the total variation in soil physicochemical parameters. Millipede-treated microcosms clustered separately from the control, reflecting overall higher concentrations of P, K, Ca, Mg, SOC, EC and elevated pH in the presence of millipedes (Table 1 and Fig 3). Samples associated with *A. gravelyi* were more widely dispersed along the principal component axes, indicating a broader and more pronounced influence on soil element distribution compared to *A. saussurii* and *Phyllogonostreptus* sp. This pattern highlights species-specific differences in the extent of soil modification driven by millipede activity. PCA results corroborate the ANOVA findings that soil properties differ significantly among microcosms.



Fig 1: Millipede species used for the present study







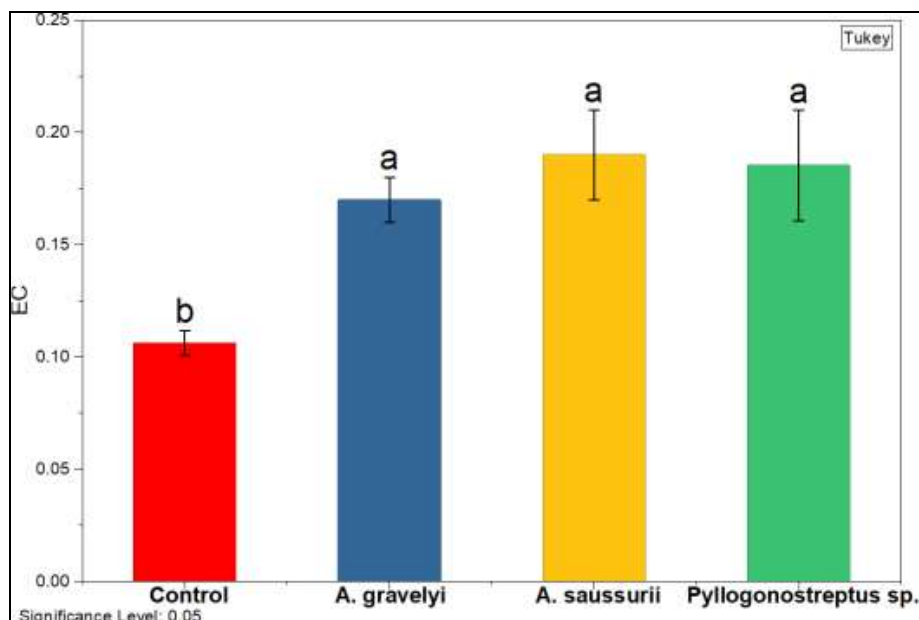


Fig 2. Results of Tukey's HSD post hoc test showing differences in soil physicochemical properties among the control and millipede treatments. Means sharing the same letter are not significantly different ($p > 0.05$), whereas means with different letters differ significantly ($p < 0.05$)

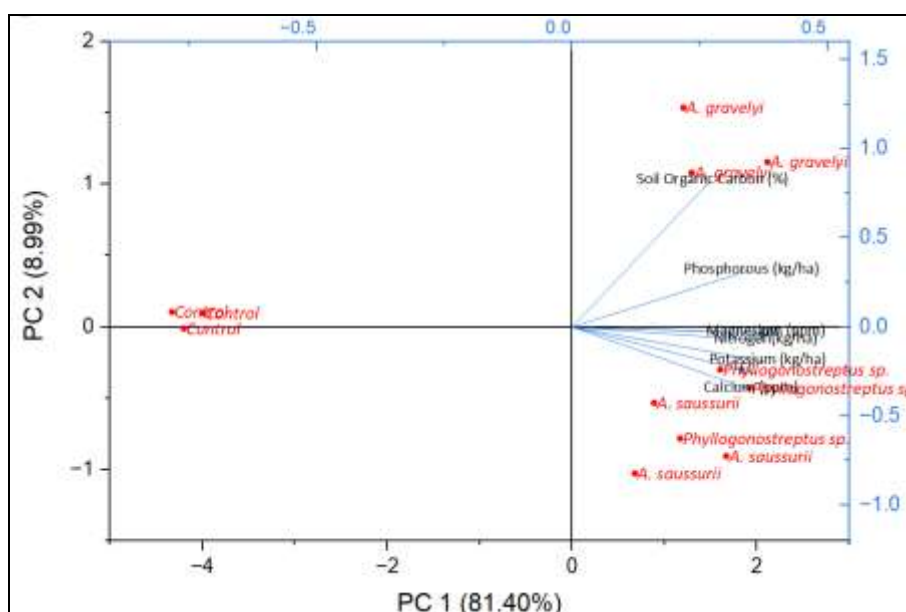


Fig 3: PCA of Soil Physicochemical Properties in Millipede Microcosms

Table 1: Principal component loadings of soil parameters for PC1 and PC2

	Coefficients of PC1	Coefficients of PC2
pH	0.383	-0.035
Soil Organic Carbon (%)	0.271	0.820
Nitrogen(kg/ha)	0.375	-0.077
Phosphorous (kg/ha)	0.347	0.316
Potassium (kg/ha)	0.379	-0.194
Calcium (ppm)	0.346	-0.347
Magnesium (ppm)	0.375	-0.032
EC	0.340	-0.246

Conclusion

This study showed that the three millipede species, *A. gravelyi*, *A. saussurii*, and *Phyllogonostreptus* sp., improved several soil properties under laboratory conditions. The activity of millipedes increased soil pH, SOC, N, P, K, Ca, Mg, and EC compared with the control soil. *A. gravelyi*

produced the highest SOC content, whereas *A. gravelyi* and *Phyllogonostreptus* sp. recorded the highest phosphorus and calcium concentrations. These results show that millipedes play an important role in litter decomposition and nutrient cycling.

The study highlights the importance of millipedes in maintaining soil fertility and soil health. Further studies under field conditions are needed to understand their long-term effects on soil properties and ecosystem functioning.

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