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The effects of feeding with organic waste by terrestrial isopod *Philoscia Muscorum* on enzyme activities in an incubated soil

Sholpan S. Muminova [a], Gulsun Bayadilova [b,*], Oryngul Mukhametzhanova [c], Saule M. Seilgazina [c], Roza Zhumabayeva [d], Gulnissam Rvaidarova [e]

[a] University named after Zhumabek Akhmetuly Tashenev, Shymkent, Kazakhstan
[b] Kazakh National Agrarian Research University, Almaty, Kazakhstan
[c] Shakarim State University of Semey, Kazakhstan
[d] M. Auezov South Kazakhstan State University, Shymkent, Kazakhstan
[e] LLP "Kazakh Research Institute of Plant Protection and Quarantine named after Zh. Zhiembaev", Almaty, Kazakhstan

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Author(s)

S.S.Muminova
G.Bayadilova*
O.Mukhametzhanova
S.M. Seilgazina
R.Zhumabayeva
G.Rvaidarova

* Corresponding author

Abstract

Soil fauna are important biological factors that affect litter decomposition and play an important role in the release of nutrients and improve soil enzyme activities. This study focused on the effects of isopods on enzymatic activities of soil. Lab experiments were conducted to assess the influence of terrestrial isopod Philoscia Muscorum on enzyme activities during the incubation. In Lab experimental food sources from wheat straw were prepared. Dehydrogenase, urease, alkaline phosphatase and arylsulphatase activity in soil treated with different number of isopods with wheat straw were determined in 28 days incubation. Results showed that the presence of isopods significantly increased ($P<0.05$) enzymatic activities of soil except arylsulphatase compared with the control treatment. The findings demonstrate that the isopods could accelerate litter decomposition and improve soil dehydrogenase, urease and alkaline phosphatase activities in soil. This work provides evidence demonstrating that soil fauna can improve soil enzyme activity by promoting wheat straw decomposition.

Keywords: Isopod, dehydrogenase, urease, alkaline phosphatase, arylsulphatase, soil.

Introduction

Litter fragmentation is the first step in the recycling of organic matter. Soil macrofauna affect litter decomposition and available nutrient contents in soil by influencing the microorganisms and their activities (Morgan and Mitchell, 1987). Isopods transport organic material deeper into the substrate. In addition, soil nutrients such as N, P and S are affected by macrofaunal activity through changes in substrate-decomposition rates and increased substrate surface area (Seastedt and Crossley Jr., 1980; Visser, 1985; Morgan and Mitchell 1987; Anderson, 1988a,b). It comprises primary attack of the protecting cuticle and epidermis and further comminution of leaves, thereby increasing the surface area of the litter particles and mixing litter particles intimately with microorganisms. These processes strongly favour microbial attack and, hence, mineralization of the organic substances from plant cells. When microbial processes taper off, renewed fragmentation or grazing by fungivores will revive microbial activity (Eijsackers, 1991). As a consequence, terrestrial isopods indirectly affect the activity and community composition of the soil microflora and their activities (Teuben and Roelofsma, 1990). The isopod *Philoscia muscorum* is a common and abundant member of the saprophagous soil macrofauna in Kazakhstan (Bragina and Khisametdinova, 2014).

The ecological function of isopods has been extensively studied, but little research has been conducted on the effects of isopods on soil enzymatic activities. Therefore, evaluating the relationship among isopods, their feds

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and soil is necessary. A comparison of enzyme activities in a soil with and without terrestrial isopods should further illuminate the effect of terrestrial isopods on enzymatic activity in soil ecosystem. The objective of the present study was to examine the effects of terrestrial isopod *Philoscia Muscorum* on enzymatic activity in loamy soil. These results will help understanding the effect of isopod Philoscia Muscorum on enzymatic activity in soil.

Material and Methods

Soil, organic waste and isopod *Philoscia Muscorum*

The surface soil (0-20 cm) used in this experiment and contained 14.45% clay, 42.65% silt, 42.9% sand. Soil texture can accordingly be classified as loam (L). The pH in water was 7.26, electrical conductivity in water was 0.11 dSm^{-1}, $CaCO_3$ content was 5.76%, the oxidizable organic matter content was 1.25%, the total N was 0.018%, $NaHCO_3$ extractable P was 5.86 mg kg^{-1}, the soil C:N ratio was 40.3. The soil was bulked, all stones, visible roots and fauna removed, sieved to less than 2 mm and stored at 5^0C until used. Wheat Straw as an organic waste was obtained from the field after wheat harvesting. The wheat straw was composed of approximately 92% by weight of oxidable organic matter. The organic fraction comprised 53% C and 0.42% N while the inorganic fraction contains 0.25% P_2O_5 and 4.77% K_2O by weight. The wheat straw in this experiment was digested and air dried and sieved to less than 1 mm and stored in polyethylene bags at 5^0C until used. Also *Philoscia muscorum* (Isopod; Philosciidae) which was used in the experiment, has been collected from the field.

Experimental procedure

On top of 50 gr air-dried soil which was contained within 150 ml glass containers, 10% (5 g) wheat straw has been added. Afterwards, the moisture content of the soil has been moistened with distilled water enough to be at field capacity level. Subsequently, on top of the soils *Philoscia muscorum* has been added in increasing numbers (0, 5, 10, 15 and 20 piece/50gr). The glass containers containing the soil, organic material and *Philoscia muscorum* has been left to incubation (25±2^oC) in the laboratory. The experiment established as 3 replications, and formed with 60 glass containers. During incubation, by weighing every day, the diminished water from the soil re-added to the medium, and properties of the soil samples which were taken in 7th, 14th, 21st and 28th days of the incubation has been determined in 3 parallels.

Measurement of soil enzymatic activities

Dehydrogenase activity was determined according to Pepper et al. (1995). Six grams of soil, 30 mg glucose, 1 ml of 3% 2,3,5-triphenyltetrazoliumchlorid (TTC) solution and 2.5 ml pure water were added. The samples were incubated for 24h at 37^oC. The formation of 1, 3, 5 triphenylformazan (TPF) was determined spectrophotometrically at 485 nm and results were expressed as μg TPF g^{-1} dry sample $24h^{-1}$.

Urease activity was measured by the method of Hoffmann and Teicher (1961)**.** 0.25 ml toluene, 0.75 ml citrate buffer (pH, 6.7) and 1 ml of 10% urea substrate solution were added to the 1 g sample and the samples were incubated for 1h at 37^0C. The formation of ammonium was determined spectrophotometrically at 578 nm and results were expressed as μg N g^{-1} dry sample.

Alkaline phosphatase activity was determined according to Tabatabai and Bremner (1969). 0,25 ml toluene, 4 ml phosphate buffer (pH,8.0) and 1 ml of 0,115 M *p*-nitrophenyl phosphate (disodium salt hexahydrate) solution were added to the 1 g sample and the samples were incubated for 1h at 37^0C. The formation of *p*-nitrophenol was determined spectrophotometrically at 410 nm and results were expressed as μg *p*-nitrophenol g^{-1} dry sample.

Arylsulphatase activity was measured according to Tabatabai and Bremner (1970). 0.25 ml toluene, 4 ml acetate buffer (pH,5.5) and 1 ml of 0.115 M *p*-nitrophenyl sulphate (potassium salt) solution were added to the 1 g sample and the samples were incubated for 1h at 37^0C. The formation of *p*-nitrophenol was determined spectrophotometrically 410 nm and results were expressed as μg *p*-nitrophenol g^{-1} dry sample.

All determination of enzymatic activities were performed in triplicate, and all values reported are averages of the three determinations expressed on an oven-dried soil basis (105 ^{0}C).

Statistical Analysis

In order to perform the ANOVA test for the results obtained from the experiments and to demonstrate the statistical differences, the LSD test has been performed with SPSS 11.0 statistical software package.

Results and Discussion

The changes in the enzyme activities of the soils during the 28-day incubation period with the addition of an increasing number of terrestrial isopod *Philoscia Muscorum* to the soils are shown in Figure 1. According to the results obtained, it was determined that the isopods added to the soils affect the activities of different enzymes in the soils differently. Isopods exert direct and indirect effects on the promotion of litter decomposition (Jia et al., 2015). Our study showed that isopod treatment can significantly (P < 0.05) promote enzymatic activities except arylsuphatase compared with control treatment. Two main reasons account for this phenomenon. First, isopods can directly feed and break down wheat straw to improve its decomposition. Second, they can indirectly change the quantity, structure, and activity of soil microbial communities (Jia et al., 2015). Macro-detritivores feed on large amounts of litter and expel the resulting organic material in the form of feces, which could reach a critical level of energy and nutrient input for soil microorganisms (Hunter, 2001; Clark et al., 2010; Yang et al., 2020). According to this results, it can be said that isopods have a greater effect on soil enzymatic activities. These effects were most profound during the incubation periods of the present experiments, when synthesis of enzymes by microorganisms are expected to be dominant.

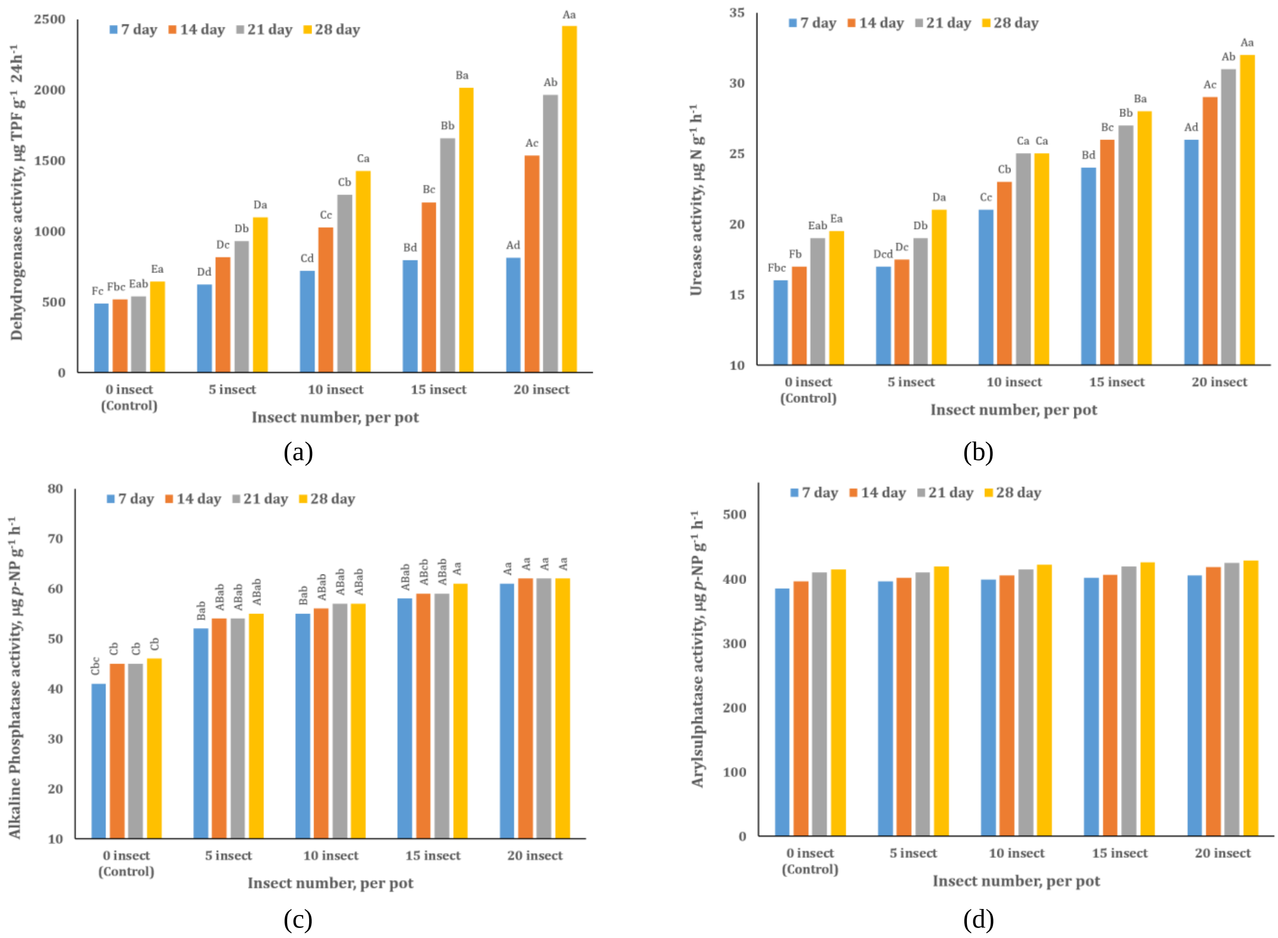

Figure 1. The impact of isopod *Philoscia muscorum* added in increasing numbers to the soil mixed with 10% wheat straw, on the soil enzyme activities (a) Dehydrogenase activity (b) Urease activity (c) Alkaline phosphatase activity (d) Arylsulphatase activity

During the incubation period, it was determined that the change in soil enzyme activities after the application of terrestrial isopod *Philoscia Muscorum* to the soil in increasing numbers with wheat straw was most evident in dehydrogenase activity (Figure 1). In the control application (0 insect), it was determined that dehydrogenase activity increased when only wheat straw was given to the soil (P<0.05). In many studies (Kızılkaya, 2008), it has been determined that the dehydrogenase activity of the soils increases when organic matter is added to the soil. However, it was determined that insect added to the soil in increasing numbers increased the dehydrogenase activity, and this increase was more in the later stages of incubation (P<0.05). The highest dehydrogenase activity was determined in soil treated with 20 insect and on the 28th day of incubation (Figure 1a). As presence of dehydrogenases, which are intracellular to the microbial biomass, is common throughout microbial species and they are rapidly degraded following the cell death, the measurement of microbial dehydrogenase activity in soils and sediments has been used extensively (Bolton Jr. et al., 1985; Rossel and Tarradellas, 1991; Obbard, 2001). Therefore, usage of dehydrogenase activity as an index of microbial activity has been suggested (Benefield et al., 1977; Masciandaro et al., 2000; Zhang et al., 2006). The increase in the dehydrogenase activity of isopod added to the soil in increasing numbers at the end of this experiment reveals that it is an important indicator that the microbial activity of the soils has increased.

It has been determined that terrestrial isopod *Philoscia Muscorum* added to soils in increasing numbers increase urease activity as well as dehydrogenase activity. It was determined that there was an increase in the urease activity of the soils by adding only wheat straw to the soil in the control soil without isopod (Figure 1b). Urease is involved in the hydrolysis of urea to carbon dioxide and ammonia, which can be assimilated by microbes and plants. It acts on carbon-nitrogen (C-N) bonds other than the peptide linkage (Bremner and Mulvaney, 1978; Karaca et al., 2002; Kızılkaya and Bayraklı, 2015). In the experiment, the highest urease activity was determined in the application with 20 insect and on the 28th day of incubation. The reason for the increase in urease activity with the addition of isopod is thought to be due to the synthesis of the urease enzyme by this microflora by the increased microbiological activity.

The change in the alkaline phosphatase activity of the soils during the 28-day incubation period of the soils with increasing numbers of isopods added with wheat straw is given in Figure 1c. According to the results, it was determined that the addition of terrestrial isopod *Philoscia Muscorum* increased the phosphatase activity of the soils compared to the control. Although there was no significant relationship between incubation period, the highest phosphatase activity was detected on the 28th day of incubation and 20 insect additions. Phosphatase is an enzyme of great agronomic value because it hydroles compounds of organic phosphorus and transforms them into different forms of inorganic phosphorus, which are assimilable by plants (Amador et al., 1997). Variations in phosphatase activity apart from indicating changes in the quantity and quality of a soil's phosphorated substrates, are also a good indicator of its biological state (Pascual et al., 1998, 2002). In this experiment, it was determined that the addition of insects to the soil was more effective than the incubation period on the change in alkaline phosphatase activity determined in different incubation periods. In addition, an increase in soil phosphatase activity was detected in the last periods of incubation in the control application without insect. With the studies carried out by other researchers (Akça and Namlı, 2015), it has been determined that organic materials added to the soil increase the phosphatase activity of the soils.

The effect of isopods added to the soil in increasing numbers together with wheat straw on the arylsulphate activity of the soils is given in Figure 1d. According to the results obtained, the effects of isopod addition on the arylsulphate activity of the soils were not found to be statistically significant. Arylsulphatase is the enzyme involved in the hydrolsis of arylsulphate esters by fission of the oxygen-sulphur (O-S) bond. This enzyme is believed to be involved in the mineralisation of ester sulphate in soils (Tabatabai, 1994). Also, it may be an indirect indicator of fungi as only fungi (not bacteria) contain ester sulphate, the substrate of arylsulphatase (Bandick and Dick, 1999). According to the results obtained, the effects of isopod addition on the arylsulphate activity of the soils were not found to be statistically significant.

Conclusion

Our results indicated that terrestrial isopod *Philoscia Muscorum* can significantly promote wheat straw decomposition and isopods could significantly improve the soil enzymatic activities of soil during the incubation. The promotion of soil enzymatic activities by the isopods was significantly affected by insect numbers, incubation time, and their interactions. This work highlights the interconnections between isopods and soil enzyme activities and the importance of further research on the ecological functions of soil fauna.

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