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Nutrient and enzyme mobilization in earthworm casts: A comparative study with addition of selective amendments in undisturbed and agricultural soils of a mountain ecosystem

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ABSTRACT

Earthworms are known to bio-degrade various types of organic materials added or already present in soil. Two soils were collected from different land use patterns viz. an agricultural land (AL) and an undisturbed land (UL) of a mountain ecosystem of State of Himachal Pradesh in India for the present investigation. A laboratory microcosm study was carried out to elucidate effects of various agricultural-soil amendments, viz. wood ash (WA), rice husk (RH), cow dung (CW) and their mixture (MA) applied @25 t ha^{-1}, on selective biological and chemical properties of earthworm (*Eisenia fetida*) casts and growth in the collected soils. Subsequently, earthworms were reared in soils from both AL & UL, treated with the amendments; and the resultant earthworm cast (E_wC) properties were found to be largely determined by the nature of amendments rather than land use types. Soils treated with MA produced casts displayed higher nutrient content, microbial biomass C with higher activities of dehydrogenase and urease. Earthworm growth and cast formation were greater in the soil from UL over soil from AL for all treatments except WA, implying better acclimatization and earthworm activity in the former. Further, casts produced under UL had higher nutrient mobilization and enzyme activity in comparison to AL. Results suggested that agricultural intervention in an undisturbed land might weaken its ability to support growth, development in conjunction with the activities of earthworms and associated enzymes.

1. Introduction

The fundamental role of earthworms in decomposition of organic matter and nutrient cycling in soils is well known and its significance in agriculture well established (Zhang et al., 2000). Nutrient enriched earthworm casts (E_wC) enhance soil fertility to great extents (Buck et al., 2000). The abundance of fulvic acids, humic acids and enzymes in E_wC enhances plant growth (Kizilkaya and Hepsen, 2004). Microbial and nutritional properties of E_wC *vis a vis* their role in soil fertility have been widely studied (Aira and Dominguez, 2009). Microorganisms in earthworm gut obtain their nourishment from the ingested organic material while earthworms promote soil microbial activity through their microorganism-rich casts (Ali et al., 2015). Microbial biomass is a key attribute of soil quality while microbial parameters are integral components of soil organic matter (SOM) and labile pool for plant nutrients. But, no single microbial parameter can be used universally (Nannipieri et al., 1990). Microbial biomass and enzyme activity measurements appear to provide more sensitive indications of soil perturbations than either activity or microbial population measurements alone. Parameters that have some form of 'internal control', e.g. microbial biomass as a percentage of soil organic matter, are also useful (Brookes, 1995).

E_wC also contributes to the soil enzymes and enhances the microbial population in soil (Kharrazi et al., 2014). Soil enzymatic

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activity is an important indicator of soil fertility and soil microbial activity (Tao et al., 2009). Soil enzymes are integral part of nutrient cycles and are active in catalysing various biochemical reactions in soil (Tabatabai, 1994). Soil enzymes along with microbial biomass are important biochemical parameters that define biological activities in soils (Kizilkaya and Hepsen, 2004). Dehydrogenase enzyme activity (DHA) is one of the important parameters that indicate microbial and metabolic activity of microorganisms (Kizilkaya and Hepsen, 2004; Tabatabai, 1994). Dissimilatory nitrate reductase (NR) enzyme mediates in the denitrification process of soil by converting NO_3^- to NO_2^-. Further, nitrite (NO_2^-) gets converted to nitrous oxide (N_2O) and finally nitrogen (N_2) is released into the atmosphere. Both NR and urease (U) enzymes play crucial roles in the nitrogen cycle. An enzyme like urease is essential for releasing mineral nitrogen (N) from soil organic matter and is highly influenced by the frequency and dose of urea applied to soil.

Appropriate and cost effective technologies can potentially offer suitable remediation processes for polluted soils and sustainable agricultural practices in developing countries (Haller et al., 2016). Therefore, for an enduring and sustainable agriculture, the use of organic wastes as amendments is a crucial area of research. In soil, degradation of organic matter by earthworms in the presence of micro flora stabilizes and converts the important nutrients into more plant available forms (Taeporamaysamai and Ratanatamskul, 2016) by a non-thermophilic process (Kharrazi et al., 2014). The muscular action of the foreguts and added mucus of earthworms help in degradation and homogenization of the organic amendments, thus facilitating microbial action over an increased surface area. Further, the degradation processes convert organic substances into casts by the accelerated action of mineralization and consequently these casts show augmented level of nitrate, phosphorus, potassium, calcium, magnesium and plant growth hormones (Taeporamaysamai and Ratanatamskul, 2016).

Soils under different land use often differ in physico-chemical and biological properties. Earthworms could significantly increase soil fertility through bio-processing of organic matter, nutrient mobilization and homogenization of soils. The effects of land use on E_wC production, their properties and their potential to act as soil quality indicators have not been studied extensively (Hauser et al., 2012). Hence, it is important to understand soil and land use patterns that could influence earthworm activities. The objectives of the present investigation were i) to study the effects of local soil amendments on enzyme and nutrient mobilization in E_wC developed in soils from an agricultural land and an undisturbed land (that may be brought under agricultural practices in future) of a mountain ecosystem and ii) to carry out a comparative assessment of the impacts of land use and amendments on E_wC properties.

2. Materials and methods

2.1. Study site and soil sampling

Soil samples were collected from an area (N 32°01.607′, E 76°42.702′) situated in the mountainous state of Himachal Pradesh in India with a maximum of 144 mm rainfall in the month of July and with annual mean minimum and maximum temperatures of 5 °C and 28 °C respectively. Soils were collected from two different land uses (i) agriculture land (AL) which was under continuous maize-wheat crop rotation for the last 50 years and (ii) an undisturbed land (UL) (which is yet to be brought under agriculture). The collected soils had silty loam texture with 29.5% sand, 46.8% silt and 23.7% clay. The soil samples were collected from a depth of 0–10 cm, air dried, sieved and stored in a dark room at 30 °C till used for further analysis.

2.2. Indigenous organic amendments as treatments

In the present experiment wood ash (WA), rice husk (RH) and cow dung (CW) which are commonly used as amendments in rural areas in the state of Himachal Pradesh for enhancing soil fertility, were used as treatments along with a control (CT; no amendment but only earthworm). Alkaline WA, generated from local firewood combustion, is customarily used to elevate pH of acidic soils whereas RH is incorporated in crop fields as a source of specific nutrients. Cow dung produced in abundance in this region from domestic cows and is applied to the crop fields after necessary sun-curing for soil fertility enhancement. A mixed amendment (MA) was also prepared by mixing all three types of amendments in 1:1:1 ratio.

2.3. Experimental design

A microcosm experiment was conducted in the laboratory with young earthworms (*Eisenia fetida*; 2.5–4 cm in length and 0.4–0.6 g in weight) which were collected from the same agricultural field from where the soil for this experiment was gathered. These fields were under local farming practices and thus the soils were incorporated with organic manures (mostly farm yard manure). One kg of soil from each land use was placed in plastic tray (20 cm × 15 cm × 8 cm) and kept away from direct sunlight. To mimic the conventional agricultural field condition, nutrients (viz. N, P, K) were applied to all the soils (including control soils) through urea, di-ammonium phosphate and muriate of potash as per the normal farming practice in the region (@120:60:60 kg ha^{-1}). The amendments viz. WA, RH, CW and MA were tested for each land use type (AL and UL) in three replicates over a period of three weeks following a completely randomized design (CRD). Two weeks prior to the addition of earthworms, amendments were applied in soils at a rate equivalent to 25 ton ha^{-1} and were kept moistened with distilled water. Twenty earthworms were added to each treatment. Soils without amendment but with earthworms were taken as controls. In control soils, due to lack of organic matter, earthworms died frequently and new earthworms were added to maintain the cast production in these soils. Every morning, over a period of three weeks, fresh E_wC were collected from the surface of the soils and composite samples were prepared for each treatment. The E_wC samples were stored at 4 °C for further analysis. Fresh E_wC were used for estimation of selected enzymes, nitrogen (total, NH_4^+ and NO_3^-) and microbial biomass carbon (MBC). Moisture level of each treatment was maintained at about 60% (w/v) by applying distilled water periodically and temperature was maintained at about 20–22 °C. After three weeks, earthworms were retrieved from the individual treatments and their live biomass was measured.

2.4. Characterization of organic amendments and earthworm casts

Morphological characterization (Fig. 1) of the selected organic amendments was undertaken by scanning electron microscope (SEM) (Zeiss, EVO 40) with an accelerating voltage of 10,000 V, working distance 9900 μm and emission current of 13,300 nA. Total carbon and nitrogen in amendments were estimated by a CHNS analyser (LECO CHNS-932); elemental composition of amendments were analysed by Energy Dispersive X-ray Fluorescence (model- P Analytical Epsilon 5). Organic carbon (OC), available phosphorous (P) and potassium (K) in casts were determined by standard methods (Page et al., 1982). Mineral nitrogen (NH_4^+ and NO_3^-) were determined by indophenol blue and phenoldisulfonic acid methods respectively (Subbiah and Asija, 1956). MBC was determined by the Fumigation Method (Vance et al., 1987). DHA in the cast was determined by the method described by Klein et al. (1971) whereas