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Original article

Quantifying earthworm species richness in the pineapple and mixed fruit plantations of West Tripura, India – A non-parametric approach

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ABSTRACT

Species richness is a fundamental measurement of community and regional diversity. In spite of its importance, ecologists have not always appreciated the effects of sampling efforts and abundance on richness measures and comparisons. The species richness, abundance of earthworms were investigated in the pineapple (PP) and mixed fruit plantations (MFP) of West Tripura, India. A total of 11 and 14 earthworm species belonging to 8 genera of 4 families were collected by conventional digging and hand sorting method from PP and MFP respectively. A comparative analysis of inventory completeness and species richness among the two types of plantations was done by using species accumulation curves and other non-parametric richness estimators. Species accumulation curves revealed more completeness of the inventory in MFP than that of PP. We use six different estimators based on abundance data: Chao 1, Chao 2, ACE, ICE, Jack-knife 1 and Jack-knife 2. For MFP, all the estimators except Jack-knife 2, showed accurate prediction of species richness; whereas only Chao 1 and Chao 2 estimators perform particularly well in case of PP.

1. Introduction

Species is the fundamental unit of biodiversity [1,2]. Hence estimating species richness is essential in biodiversity studies [3]. In fact, taxon inventory of a site is a challenge to ecologists because it is rarely possible to collect enough individuals to discover total species present in a particular community or area [4]. Therefore, observed number of species is a biased estimate of community species richness; because observed richness is usually lower than the true species richness [5].

The difficulty of inventories based on repeated sampling is that irrespective of number of sampling units and methods used, the result will be either large number of species with few individuals or small number of species with many individuals [6]. Since a substantially incomplete study resembles very much with a complete study, the question that rises is how we can appreciate further effort and required time because of the limiting factors like time constraint, financial and personnel resources of biodiversity research [7]. Therefore, description of species abundance distribution followed by an extrapolation of species richness is essential for biodiversity studies [8–10]. Many mathematical and statistical models were established to describe species abundance distribution of a given area [3]. Despite the wider use of these models in biodiversity studies, in recent days [11–14], these are not usually used in earthworm research [15].

Several reasons are there behind selecting earthworms as the taxon for survey. Being the soil dwelling invertebrate macro-fauna with highest biomass in tropical soil [16], they have a large impact on soil quality by their peculiar feeding, burrowing and casting activities [17]. Since, earthworm species composition and population structure is sensitive to changes in the land use pattern and plantation type [18–20], they are useful bio-indicators in soil ecosystem studies [21].

Now-a-days, ecological studies on earthworms are very common but in most cases are results of small scale sampling. Earthworm communities are generally species poor in a given ecosystem [15] comprising of only 4–14 species [21]. So it is difficult to perform an exhaustive biodiversity survey for earthworms in a given community [22]. In addition, variations in the characteristics of biological assemblages produce differences in sampling effectiveness [23].

Owing to this, the aim of our present study is to establish a methodological and theoretical frame work for the application of species accumulation curve, sample-based rarefaction curves and non-parametric richness estimators in earthworm biodiversity research and to combine information on alpha diversity with

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ecological patterns of organisms (e.g. spatial distribution and substrate specificity). Thus we focused on the following aspects:

(1) Usefulness of non-parametric species accumulation and sample-based rarefaction curves for determining optimum sample size to reach an asymptotic richness.
(2) Efficiency of non-parametric richness estimators to estimate earthworm species richness in pineapple and mixed fruit plantation.

2. Materials and methods

2.1. Study sites

The present paper is based on the earthworm survey data obtained during April 2008–October 2010 in the pineapple (PP) and mixed fruit plantations (MFP) in West Tripura (22°51′–24°32′N and 90°10′–92°21′E). Three study sites were considered for each type of plantation i.e. pineapple (PP) and mixed fruit plantation (MFP). The study areas experienced a tropical climate with a mean annual rainfall of 2000 mm and temperature of 25 °C. Soils of Tripura are acidic (pH 4.6–5.5) in general and have developed from non-calcareous parent material [24].

The sites employed for earthworm surveys were 30–35 year-old plantations. Prior to their conversion to PP and MFP, these sites were mainly fallow lands without any agricultural activity. The distance between the sites ranged from 20 to 50 km. Because pineapple plants cannot stand water-logged conditions, they are usually planted on undulating uplands. The well-drained soils were acidic with sandy loam or silty loam texture. Along with different weeds (herbs and shrubs), PP had a low canopy cover comprised of a few scattered trees of litchi (*Nephelium litchi*: Sapindaceae). MFP by contrast had a good canopy cover over the pineapple crop, consisting of a number of fruit tree species.

2.2. Earthworm sampling

Earthworms were collected during summer (April–June), monsoon (July–September) and post-monsoon period of autumn (October) of each year during the study period (2008–2010) by digging (25 cm × 25 cm × 40 cm) and hand-sorting of soil [25]. A total of 40 widely separated 10 m × 10 m plots were randomly selected for sampling in each study site. Composite soil sample comprising of five sub-samples was taken from each plot. Sampling points were located at the corner and center of each sampling plot. Thus, a total of 120 samples (600 sub-samples) were taken each from PP and MFP. In the field earthworms were counted and weighted on an electronic balance. Results were expressed in terms of density (ind m^{-2}) and relative abundance. Ecological categories and identification of earthworms were based on Bouche's classification [26] and keys provided by Gates [27] and Julka [28].

2.3. Species accumulation curves (SAC) and sample-based rarefaction curves (SRC)

Sample-based data were used for calculation of species accumulation and rarefaction curves. Species accumulation curves (SAC) are the plot of expected number of detected species as a function of sampling effort and arise as a graphical representation of the sampling process [29,30]. These curves, also been used by ecologists to perform quantitative comparison among species assemblages move from left to right, as new species are added [3,4,29]. The statistical expectation of the corresponding species accumulation curves are expressed as sample-based rarefaction curves (SRC), which themselves record the total number of species revealed as additional sample units are added in one particular random order to the pool of all previously collected samples [15,31]. Since the sample-based rarefaction curves (SRC) depend on the spatial distribution of individuals, as well as, the size and placement of the samples, it cannot be derived theoretically [4]. Both these curves are classic but informal way to assess the completeness of an inventory [32,33]. These curves may also provide an estimate of the total species richness of an assemblage, unless sampling has been exhaustive [3].

2.4. Non-parametric methods for estimating species richness

For the calculation of non-parametric richness estimators, sample-based data were used with ESTIMATES 8.2.0 [34] and graphs were generated by ORIGIN 6.0 Professional [35].

A total of six richness estimators were compared based on two types of data viz. incidence and abundance based data [36] in order to verify which one fits best to the present data set. Estimators like Chao 1 [37], abundance based coverage estimator (ACE) [38] and first order Jackknife richness estimators (Jackknife 1) [39,40] are based on abundance data, whereas Chao 2 [41], incidence based coverage estimator (ICE) [38] and second order Jackknife richness estimators (Jackknife 2) [39,40] are based on incidence data. Detailed descriptions of these algorithms are available in Colwell and Coddington [29], Toti et al. [42] and Colwell [43].

3. Results

A total of 16 earthworm species belonging to 8 genera and 4 families were collected from the study sites (Table 1). Densities and relative abundances of the above mentioned earthworm species in PP and MFP are shown in Fig. 1. Details of site characteristics, community composition, inter-habitat variations in spatial distribution and community organization of two studied sites are described elsewhere [17,44].

The species accumulation curves and sample-based rarefaction curves (Fig. 2a, b) generated from the complete sampling revealed the observed species richness (S_{obs}). Initially both the curves rose rapidly but the rate of rise slowed down later on. In PP, (eleven observed species; Fig. 2a, b) both the SAC and SRC continued to rise as sample number increased up to 120 samples with the signs approaching an asymptote. Unlike PP, both the curves (SAC and SRC) in MFP reached an asymptote, well before total accumulated sampling efforts. Steeper SAC and SRC were observed for MFP in contrast to PP.

In PP, the incidence based Jackknife 2 estimator fails to provide reasonable estimate as it generates a total species richness estimate (9.0) (Fig. 3b) that is lower than the observed number of species [45,46]. The Chao 1 estimator curve closely resembles the SRC (Mao Tau) initially but appears to level off in middle and finishes equally with the observed richness (Fig. 3a). ACE, Jackknife 1 and ICE curves on the other hand, continue to rise with increase in number of samples and begin to fall as the maximum number of samples is approached (Fig. 3a, b). Unlike the other estimators, Chao 2 stabilizes much before the total accumulated sampling efforts compared to SRC (Mao Tau) and finishes at estimating 11 species (Fig. 3b). This lack of consensus among the estimators is echoed in their total richness which do not cluster tightly but range from 9 (Jackknife 2) to 12 (Jackknife 1 and ACE). Taking into consideration of these curves, it appears that, another one earthworm species may yet to be seen in the PP.

In the MFP (14 observed species; Fig. 4a, b) the SAC reaches an asymptote. This condition eliminates the need for non-parametric estimators but at the same time allows for a direct and rigorous test