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Allometric equations for estimating fresh biomass of five soil macroinvertebrate species from neotropical agroecosystems

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ABSTRACT

Accurate estimation of soil macroinvertebrate fresh biomass is crucial to link macroinvertebrate community to ecosystem functions, but remains a challenging task under field conditions. Here, we present allometric equations to estimate the fresh biomass of three diplopods (Rhinocricidae), one earthworm (Glossoscolescidae) and one earwig species (Anisolabididae) that are abundant in soil communities and potentially important for the provision of soil ecological functions in tropical agroecosystems. Body length, body width, and body volume, were measured using a novel method of image analysis, and then used to estimate the fresh biomass. Our results show that length-biomass allometric relationships provide reliable estimation of fresh biomass for diplopods ($r^2 = 0.98$) and earwigs ($r^2 = 0.97$). However, the biomass of earthworms was not as accurately predicted by body length ($r^2 = 0.82$). The use of body volume, estimated with body length and width, allowed to increase the predictive power for earthworms. Furthermore, a general allometric equation based on body volume, including all taxa considered in this study, was found to predict 96% of the observed body weight variability, suggesting that this equation could be generalizable to a large range of soil macroinvertebrates. Therefore, we conclude that using body volume could provide a better accuracy in estimating soil macroinvertebrate biomass. Although the estimation of body volume on each individual requires an additional measure, the use of image analysis software renders this step feasible for a large number of individuals. By improving the feasibility of trait measurements, this method may facilitate field surveys and foster trait-based studies on soil macroinvertebrates.

1. Introduction

Soil macroinvertebrates are of major importance for the functioning of natural and cultivated ecosystems [1,2]. Litter transformers for instance, by consuming large amounts of litter, participate to the fragmentation of organic matter and nutrient cycling. In turn, earthworms, which are ecosystems engineers, benefit from this fragmentation and mix organic matter with mineral soil thus influencing soil structure and nutrient dynamics. Predators feed on various soil macroinvertebrate participating in population regulation and top-down control over primary consumers such as detritivores or herbivores. The activity of all those macroinvertebrates depends on the abundance and more specifically on the biomass of the community [3,4]. Therefore, those parameters are crucial to link macroinvertebrate community to ecosystem functions. Litter consumption by macroarthropods, for instance, is a biomass-dependent physiological process that is typically expressed per unit of fresh biomass [5,6]. Adequate estimation of biomass in the field is thus required to scale up from physiological processes to ecosystem functions [7].

However, while abundance is relatively easy to measure, the biomass is more difficult to determine. Among the several methods used to estimate the live biomass, the direct measurement is accurate but requires to keep individuals alive or frozen between collection in the field and laboratory measurement, which is tedious and not always feasible depending on the amount of organisms collected, the accessibility of the locations and the distance to the laboratory. Alternatively, it is possible to estimate the average biomass of individuals per species on a subsample and then multiply it by the number of observed individuals of each species. However, this procedure does not reflect intraspecific weight

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variability which can be important especially for large species covering a wide range of body weight over their life span. In this respect, allometric equations offer a good tradeoff between accuracy and endeavor. This method consists in estimating the biomass of an organism from measurements of whole or parts of the body that are easier to obtain. This approach has been proven efficient in plant ecology to estimate plant biomass with trunk diameter [8], in stream ecology to estimate macroinvertebrate biomass with body length [9] and for aboveground insects [10]. However, it has rarely been used for soil macrofauna (but see Refs. [11,12]). In the last decades, studies on earthworm species from various ecosystems used body length and preclitellar diameter in allometric equation to estimate dry body weight and reported predictive powers ranging from $r^2 = 0.35$ to $r^2 = 0.99$ [13–15]. However, there is currently no general agreement on which measure leads to the best prediction. Jiménez et al. [13] found high predictive power using body width (preclitellar diameter) while Greiner et al. [15] found that body length predicted biomass more accurately. On the other hand, they reported an increase in the quality of relationship with increasing taxonomic resolution, indicating that using a relationship for each species, or at least for each family, provides more accurate estimates. Such differences among earthworm species or families likely results from variability in body shape, as was previously reported for collembola [16]. To circumvent this issue, using body volume to estimate the biomass appears as a promising approach for soft-bodied macroinvertebrates, as suggested by the study of Berg [17] on diptera larvae.

In this study, we aimed at determining which of body length, body width, and body volume best predicts soil macroinvertebrate biomass, with allometric equations, for different soil macroinvertebrates with contrasted anatomies. Additionally, because measuring body characteristics can be tedious when animals move or stay fixed in twisted positions and because transporting animals from the field to the laboratory can be complicated, we aimed at developing a simple method to measure macroinvertebrate body characteristics from images that can be obtained in the field, using an image analysis software. The benefit of this approach is that it minimizes animal manipulation and allows to perform measures on living organisms. Body length and width were measured with this approach, while body volume was calculated from these measurements. This work was conducted on five macroinvertebrate species belonging to the Neotropical fauna that were collected in agroecosystems of Martinique (Lesser Antilles). The five species investigated included one earthworm, three diplopods and one earwig species that are all locally abundant in soil macrofaunal communities and play an important role in the provision of soil ecological functions.

2. Materials and methods

2.1. Animal collection

All individuals were collected in sugarcane fields located in Martinique (Lesser Antilles; 14°45′09.0″N 61°10′13.1″W; altitude range 10–240 m asl). The climate is tropical with an annual temperature of 26.6 °C and a mean annual precipitation of 2000 mm (1981–2010). Fields are located on the slopes of the Mount Pelée volcano where soils, derived from andesitic volcanisms, are young and sandy. Individuals were collected in the context of a wider study on soil biodiversity under sugarcane cultivation. For the purpose of this study, all soil macroinvertebrates were collected through hand sorting of 25 × 25 cm soil cores, kept in plastic vials and brought alive to the laboratory for measurements. This study presents allometric relationships on the five most abundant species of soil macrofaunal communities under sugarcane plantation of Martinique. These five species investigated belong to three taxonomic groups: earthworms (Family: Glossoscolescidae), with *Pontoscolex corethrurus* (Mueller); iuliform millipedes (Family: Rhinocricidae) with *Anadenobolus monilicornis* (Von Porat, 1876), *Anadenobolus leucostigma* (Pocock, 1894) and *Trigoniulus coralinus* (Gervais, 1847); and earwigs (Family: Anisolabididae), with *Euborellia caraibea* (Hebard, 1921).

2.2. Animal measurements

Measurements were made the same day as collection. For each individual, a picture was taken, in the same box, with a scale bar of 10 mm on the bottom. The picture should be taken always at the same distance and most importantly perpendicular to the bottom of the box. In the present study, the lens of the camera fitted the diameter of the box so that pictures were always taken at a distance of 13.8 cm separating the bottom of the box and the sensor of the camera. The camera used was a Panasonic Lumix DMC-FZ200 with a resolution of 12.1 Megapixels. With such configuration, parallax error could arise making the object closer to appear larger [18] and because the scale bar was set at the bottom of the box, the size of the biggest animals that we measured could have been overestimated. Objects with sizes ranging from 1.5 to 6.5 mm height were used to make a calibration curve, estimate parallax error and correct the row data. The relationship between object height and parallax was found to be exponential and follow the equation: $\textit{Parallax error}\ (\%) = 3.87^{\,1.5 \times height}$. Width and length values were then corrected according to this relationship (Fig. A3). Before taking the picture, earthworms were rinsed with distilled water, to get rid of adhering soil particles, and then slightly drained using absorbent paper. Although it has been recognized that desiccation can be a source of error when measuring fresh mass, as compared to dry mass [19], the present work focused on fresh weight of macroinvertebrates as our aim was to develop a non-destructive method that can be performed in field conditions. The fresh weight referring to each individual was then recorded using laboratory scale (±0.1 mg). Size measurements were made on images using image analysis software (ImageJ, version 1.46r). For each image, scale bar length was recorded (in pixel) so that each measure was individually calibrated (see Fig. 1). For body length measurements, attention was payed to ensure that animals laid at the bottom of the box, and then the distance between the two extremities of the animal was measured. When animals were twisted or rolled up, the segmented lines method were used to measure length. Body width measurements consisted in measuring the width at a given point of the body. For earthworms it was made before the clitellum (preclitellar diameter) according to the same method as Greiner et al. and Jiménez et al. [13,15]. Width measures on immature individuals were made at the place where the clitellum should develop, i.e. approximately between the 10th and the 15th body segment. For diplopods, width measures were made between the 5th and the 10th body segment and for earwigs, they were made at the metathorax level. Assuming that earthworms and diplopods had a tubular body, we estimated their body volume using the following formula: $Volume = \pi \times \left(\frac{width}{2}\right)^2 \times length$. As earwigs body is not cylindrical, its volume was calculated with the cuboid formula. As the height could not be measured directly from the images, we estimated a height/width ratio of 0.67 ± 0.04 on a subset of five individuals. Only complete individuals were used for allometric relationships, while fragmented individuals were omitted.