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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: $\textit{Parallax error}\ (\%) = 3.87^{\,1.5 \times \textit{height}}$. 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: $\textit{Volume} = \pi \times \left(\frac{\textit{width}}{2}\right)^2 \times \textit{length}$. 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.

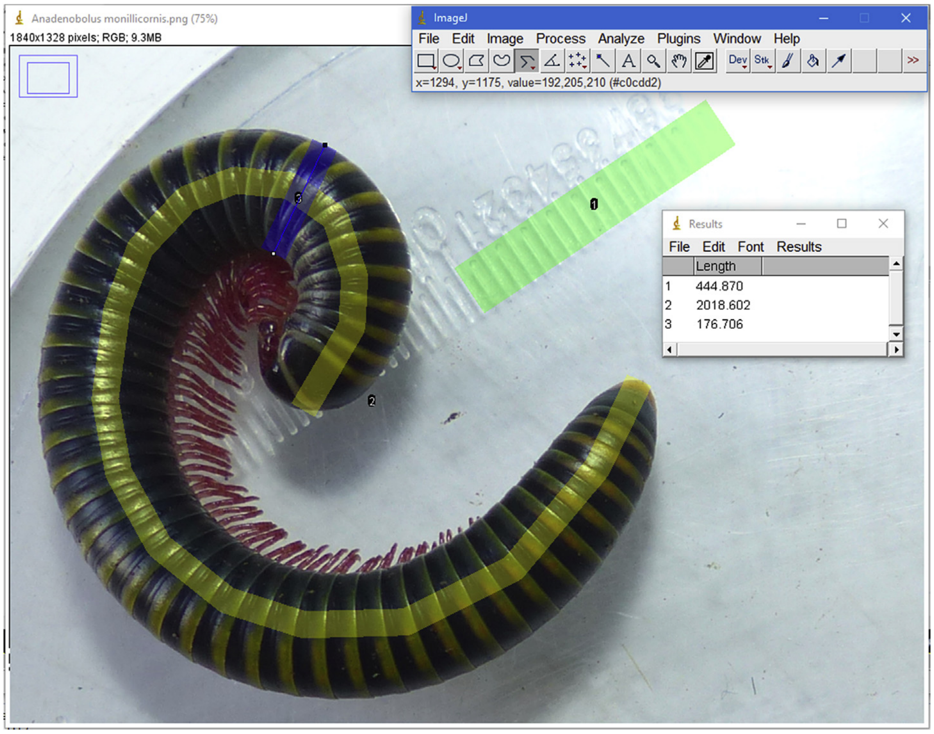

Fig. 1. Illustrated protocol of size measurement (a diplopod shown as an example) using ImageJ software. For each image, corresponding to an individual, three measurements were made simultaneously. First, length of the bar scale was recorded (in green) to make the conversion between image measurements in pixel and real length in mm. Second, the body length was recorded (in yellow) by measuring the length between anterior and posterior end of the macroinvertebrate. Third, the body width was recorded (in blue) by measuring the length between the 5th and the 10th segment of the diplopods (See text for width measurement on other species). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

2.3. Statistical analyses

The equation parameters between biomass and size measurements (i.e., body length, body width and body volume) were estimated using ordinary least square regression (OLS). Natural logarithmic transformations, which base is the *e* constant, were applied to both variables prior to analyses. Allometric relationships were first investigated for all taxa grouped together and then for the three main taxa separately (diplopods, earthworms, earwigs). Differences in slopes were tested using analysis of covariance (ANCOVA). For diplopods, allometric relationships were investigated on the three species grouped together and species-specific relationships were performed only when ANCOVA detected a significant difference in slopes. All analyses were made using R software [20].

3. Results

3.1. Length-biomass relationships

For all five species investigated, the observed development stages ranged from early stadiums to sexual maturity, thereby covering the full range of body size (Table 1) over each species life cycle. The relationship between fresh weight and body length was significant when all taxa were grouped (Table 1). This general relationship had a coefficient of determination of $r^2 = 0.75$. However, ANCOVA shows that relationships were significantly different between the three main taxa investigated ($F_{2,234} = 11$, $p < 0.001$). For diplopods and earwigs, this body-length based allometric equation explained high percentages of variability, with determination coefficient of $r^2 = 0.98$ and $r^2 = 0.97$, respectively (Table 1). For earthworms, the variability explained was lower with a coefficient of determination of $r^2 = 0.81$. Inside the diplopod taxa, there was also a significant difference between the three species ($F_{2,78} = 5$, $p = 0.007$) corresponding to small differences in slopes (Table 1, Fig. 2, Fig. A2 in appendix).

3.2. Width-biomass relationships

The relationship between fresh weight and body width was significant when all taxa were grouped (Table 2), and had a coefficient of determination of $r^2 = 0.80$. ANCOVA showed that the relationships were significantly different between the three main taxa investigated ($F_{2,234} = 5$, $p = 0.008$). Similar to the relationship with body length, the relationship between fresh weight and body width explained lower variability for earthworms ($r^2 = 0.84$) compared to diplopods and earwigs ($r^2 = 0.95$ for both taxa). However, differences in slopes between the three main taxa were less pronounced (Fig. 3, Table 2, Fig. A1 in appendix) and the relationships were not significantly different between the three diplopod species ($F_{2,78} = 0.2$, $p = 0.86$).

3.3. Volume-biomass relationships

The relationship between fresh weight and body volume was significant when all taxa were grouped (Table 3). ANCOVA showed that the relationships were not significantly different between the three main taxa investigated ($F_{2,234} = 0.2$, $p = 0.8$) and the relationship with all taxa grouped led to a consistently higher predictive power when using body volume compared to the other measurements ($r^2 = 0.97$). Furthermore, differences in slopes between the three main taxa were less pronounced (Fig. 4, Table 3, Fig. A1 in appendix) and the relationships were not significantly different between the three diplopod species ($F_{2,78} = 0.6$, $p = 0.5$).

Table 1
Equation parameters of allometric relationships between body length and body weight of the five macroinvertebrate species. All relationships shown are significant at $p < 0.001$.

Taxonomic level	*n*	Weight in mg (Min.–Mean–Max.)	Length in mm (Min.–Mean–Max.)	Equation	*F*-statistic	r^2
Diplopods (Family: Rhinocricidae)	84	1.6–162.5–852.8	3.47–23.0–50.0	ln(weight) = 2.38*ln(length)-2.77	$F_{1,82} = 3505$	0.98
Anadenobolus lecostigma	13	3.8–213.6–690.8	5.0–20.8–43.9	ln(weight) = 2.58*ln(length)-3.15	$F_{1,11} = 1515$	0.99
Anadenobolus monilicornis	40	10.4–111.7–852.8	8.4–20.0–50.0	ln(weight) = 2.47*ln(length)-3.05	$F_{1,38} = 986$	0.96
Trigoniulus coralinus	31	1.6–206.6–525.6	3.5–27.8–44.9	ln(weight) = 2.30*ln(length)-2.60	$F_{1,29} = 2276$	0.99
Earthworms (Family: Glossoscolescidae)						
Pontoscolex corethrurus	138	3.8–139.9–429.9	9.9–43.3–95.1	ln(weight) = 2.12*ln(length)-3.32	$F_{1,136} = 572$	0.81
Earwigs (Family: Anisolabididae)						
Euborellia caraibea	18	1.0–11.9–27.0	4.3–8.4–12.8	ln(weight) = 3.34*ln(length)-4.95	$F_{1,16} = 540$	0.97
All taxa grouped	240	1.0–138.2–852.8	3.5–33.6–95.1	ln(weight) = 1.72*ln(length)-1.47	$F_{1,238} = 723$	0.75

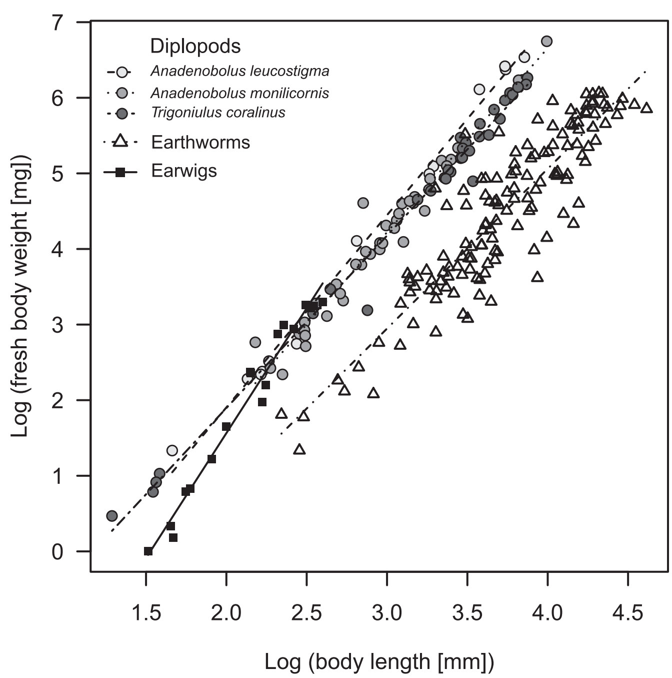

Fig. 2. Allometric relationships between body fresh weight and body length for the five macroinvertebrate species. Equation parameters are given in Table 1.

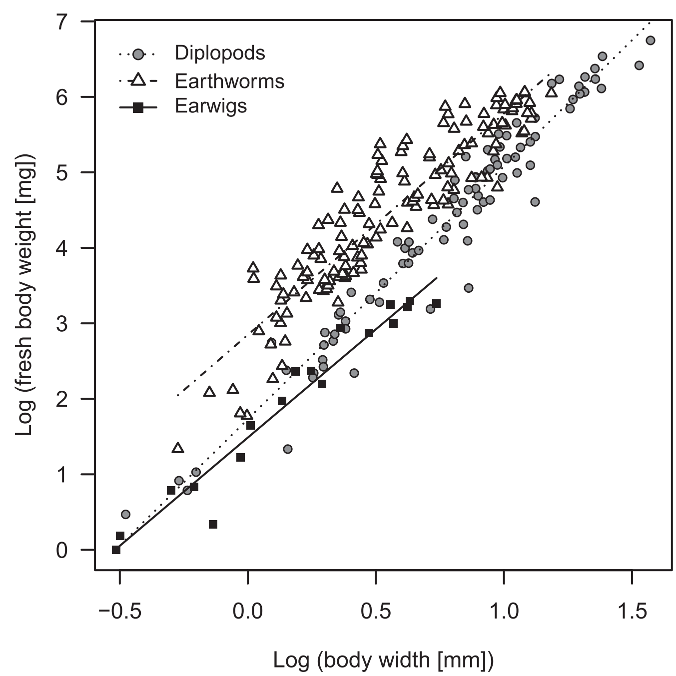

Fig. 3. Allometric relationships between body fresh weight and body width for the three macroinvertebrate taxa. Equation parameters are given in Table 2.

Table 2
Equation parameters of allometric relationships between body width and body weight of the three macroinvertebrate taxa. All relationships shown are significant at $p < 0.001$.

Taxonomic level	*n*	Width in mm (Min.–Mean–Max.)	Equation	*F*-statistic	r^2
Diplopods (Family: Rhinocricidae)	84	0.6–2.3–4.8	ln(weigth) = 3.35*ln(width)+1.73	$F_{1,82} = 1500$	0.95
Earthworms (Family: Glossoscolescidae)	138	0.8–1.8–3.3	ln(weigth) = 2.93*ln(width)+2.85	$F_{1,136} = 730$	0.84
Earwigs (Family: Anisolabididae)	18	0.6–1.3–2.1	ln(weigth) = 2.87*ln(width)+1.49	$F_{1,16} = 294$	0.95
All taxa grouped	240	0.6–2.0–4.8	ln(weigth) = 3.07*ln(width)+2.38	$F_{1,238} = 984$	0.80

4. Discussion

4.1. Relationship with a single measurement (length or width)

For diplopods and earwigs, a single measure of body length allowed to accurately predict the fresh weight ($r^2 = 0.97$ and $r^2 = 0.98$ respectively). In turn, for earthworms, the predictions of fresh weight based on a single measurement (either length or preclitellar diameter) were less accurate than for the other taxa. Low predictive powers were previously reported by Greiner et al. [15] for the tropical earthworm *Perionyx excavatus* (body length: $r^2 = 0.4$; preclitellar diameter: $r^2 = 0.3$). An explanation of these results is the distortion of earthworm body that may complicate measurements on living organisms as well as on individuals fixed in alcohol. Even if earthworms tend to curl up in alcohol, such behaviour could be species-specific, making interspecific relationships less accurate. Preclitellar measurements were initially used to overcome this bias because the gizzard, an inner organ made of thick muscles used for food fragmentation, is located in the preclitellar zone. Therefore, the size variability is assumed to be lower in this part of the body [13]. Our results showed that the use of preclitellar diameter effectively increase the predictive power of

Table 3
Equation parameters of allometric relationships between body volume and body weight of the three macroinvertebrate taxa. All relationships shown are significant at $p < 0.001$.

Taxonomic level	*n*	Volume in mm^3 (Min.–Mean–Max.)	Equation	*F*-statistic	r^2
Diplopods (Family: Rhinocricidae)	84	1.0–152.5–910.9	ln(weigth) = 1.00*ln(volume)-0.01	$F_{1,82} = 4460$	0.98
Earthworms (Familly: Glossoscolescidae)	138	5.8–175.4–692.9	ln(weigth) = 0.99*ln(volume)-0.18	$F_{1,136} = 3043$	0.96
Earwigs (Family: Anisolabididae)	18	1.0–12.4–33.7	ln(weigth) = 1.02*ln(volume)-0.08	$F_{1,16} = 521$	0.97
All taxa grouped	240	1.0–155.1–910.9	ln(weigth) = 0.98*ln(volume)-0.03	$F_{1,238} = 7766$	0.97

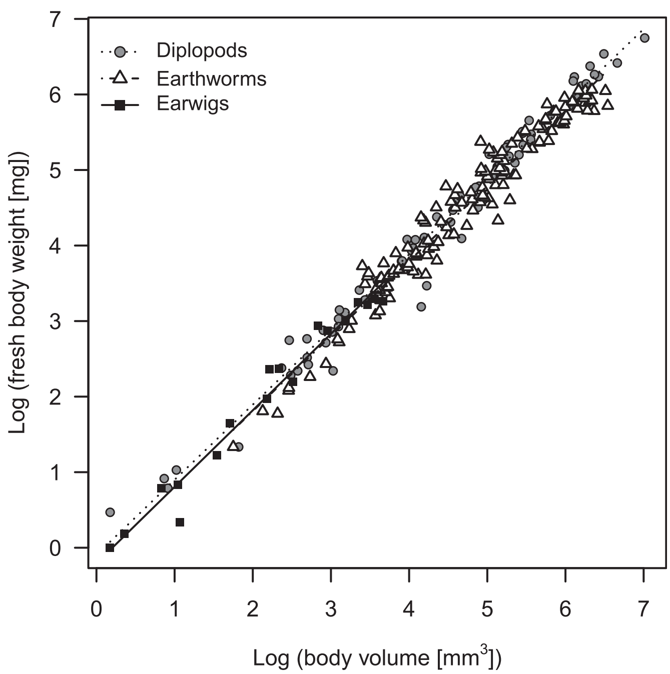

Fig. 4. Allometric relationships between body fresh weight and body volume for the three macroinvertebrate taxa. Equation parameters are given in Table 2.

the regression but the gain was not very high, as the percentage of variability explained increased from 81 to 84% only.

4.2. Taxonomic resolution of allometric equations

Whether allometric equations are more accurate when defined at earthworm species-level, as previously reported by Greiner et al. [15], could not be verified in our study as only one species of earthworm was investigated. Nevertheless, the fact that equations differed between taxa and also between diplopods species indicates that species-specific calibrations are needed to predict fresh biomass based on body length. Remarkably, equations based on body width differed notably less between taxa, and did not differ between diplopods species, suggesting that it is more robust to interspecific variability, than body length. Furthermore, as expected based on Berg's findings on diptera larvae [17], the use of volume to estimate macroinvertebrates fresh weight increased the percentage of variability explained. This result indicates that tiny differences between species can be captured using the volume, allowing to establish more generalizable allometric relationships. Strikingly, when including all taxa of this study in a general allometric equation with body volume (Table 3, Fig. 4), the biomass was accurately predicted despite the substantial differences in body shapes between taxa. The body volume thus appears as a valid predictor of fresh biomass even between phylogenetically distant macroinvertebrates.

4.3. Method of image collection and analysis

This method using images that can be taken directly in the field provides a dramatic advantage compared to direct measurements that requires transporting the animals to the laboratory. With cleaning and photographing being the only two steps requiring the animals (Fig. 1), this method may facilitate field inventories with large numbers of sampling locations. As this method is non-lethal and required no animal transportation, it is particularly suitable for studies on locations where sampling is difficult due to limited accessibility or impossible due to legal restrictions. Moreover, this method may be useful to measure other traits on fragile specimen from museums. The method of image analyses using imageJ (Fig. 1) allowed to successfully improve the efficiency of biomass estimation. Although measuring two variables on each individual represents a supplementary work, it is offset by the use of image analyses software that is less time consuming than manual measurements.

4.4. Importance of biomass estimation

Our results show that allometric equations using body length, body width and particularly body volume provide good estimations of individual fresh biomass of soil macroinvertebrates. Such methodology allows to accurately estimate the biomass of soil community, which is key to link macroinvertebrate communities to key ecosystem functions such as decomposition [21], bioregulation [22] or water infiltration [7]. Furthermore recent studies highlighted the importance of body length itself as a functional trait to predict the effect of macroinvertebrate community on ecosystem functioning [19]. Rusch et al. [22] for instance, found that predator body size could accurately predict aphid predation rate in an agroecosystem. The allometric equations defined on the five macroinvertebrate species considered in our study may help to estimate their roles on agroecosystems functioning. The peregrine earthworm *Pontoscolex corethrurus* has a pantropical distribution; it is one of the most common endogenous earthworm, and its effects on soil nutrient and nematode bio-regulation is acknowledged on many tropical agroecosystems [23–27]. Among the three diplopods species, *Trigoniulus coralinus* has a pantropical distribution and the two other species are widely distributed in the Caribbean region, playing important role in litter fragmentation in semi-natural and cultivated ecosystems [24,28,29]. Finally, the earwig *Euborellia caraibea* occurs in the Lesser Antilles where it is investigated for its ability to regulate the banana weevil population [30,31], a major pest. Combining these biomass-dependent functions with biomass estimates based on allometric equations could allow estimating the importance of these functions at large scale.

5. Conclusions

In conclusion, we advocate that body volume, estimated from image analyses, provides a better accuracy in estimating soil macroinvertebrate biomass and allows more generalizable allometric relationships, compared to body length or width. By improving the efficiency of traits measurement, this method could facilitate large field studies and foster trait-based approaches on soil macro-invertebrates. More data on biomass, length and width must be gathered on other species and taxa for more predictive and more generalizable allometric equations. For now, a calibration phase with direct biomass measurements is required to have an overview of the different morphological type susceptible to lead to contrasted relationships. However, the allometric equation established here for diplopods should be applicable to other species belonging to the orders Spirobolida, Spirostreptida and Julida which have a similar iuliform body shape.

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Appendix A

a.

```
> allsp<-aov(logb(Biomass..fresh.)~logb(Body.length..mm.) *taxa,data=Tableau.R)
> summary(allsp)
                           Df Sum Sq Mean Sq F value  Pr(>F)    
logb(Body.length..mm.)      1  342.7   342.7 2329.22 < 2e-16 ***
taxa                        2   75.2    37.6  255.46 < 2e-16 ***
logb(Body.length..mm.):taxa 2    3.2     1.6   10.86 3.1e-05 ***
Residuals                 234   34.4     0.1                    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
>
```

b.

```
> allsp<-aov(logb(Biomass..fresh.)~logb(body.width..mm.)*taxa,data=Tableau.R,)
> summary(allsp)
                           Df Sum Sq Mean Sq  F value  Pr(>F)    
logb(body.width..mm.)       1  366.8   366.8 2474.194 < 2e-16 ***
taxa                        2   52.5    26.3  177.096 < 2e-16 ***
logb(body.width..mm.):taxa  2    1.5     0.7    4.996 0.00751 ** 
Residuals                 234   34.7     0.1                     
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

c.

```
> allsp<-aov(logb(Biomass..fresh.)~logb(body.volume)*taxa,data=Tableau.R)
> summary(allsp)
                       Df Sum Sq Mean Sq  F value   Pr(>F)    
logb(body.volume)       1  442.0   442.0 9841.762  < 2e-16 ***
taxa                    2    3.0     1.5   33.611 1.47e-13 ***
logb(body.volume):taxa  2    0.0     0.0    0.191    0.826    
Residuals             234   10.5     0.0                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Fig. A1. Summary statistics of ANCOVA testing the differences in slope between the five macroinvertebrate species for lenght-biomass (a), width-biomass (b) and volume-biomass relationships (c).

a.

```
> diplosp<-aov(logb(Biomass..fresh.)~logb(Body.length..mm.) *sp,data=Tableau.R)
> summary(diplosp)
                          Df Sum Sq Mean Sq  F value   Pr(>F)    
logb(Body.length..mm.)     1 173.81  173.81 4557.658  < 2e-16 ***
sp                         2   0.69    0.34    9.009 0.000302 ***
logb(Body.length..mm.):sp  2   0.41    0.20    5.312 0.006875 ** 
Residuals                 78   2.97    0.04                      
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

b.

```
> diplosp<-aov(logb(Biomass..fresh.)~logb(body.width..mm.)*sp,data=Tableau.R,)
> summary(diplosp)
                         Df Sum Sq Mean Sq  F value Pr(>F)    
logb(body.width..mm.)     1 168.66  168.66 1538.241 <2e-16 ***
sp                        2   0.63    0.32    2.879 0.0622 .  
logb(body.width..mm.):sp  2   0.03    0.02    0.153 0.8587    
Residuals                78   8.55    0.11                    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

c.

```
> diplosp<-aov(logb(Biomass..fresh.)~logb(body.volume)*sp,data=Tableau.F
> summary(diplosp)
                     Df Sum Sq Mean Sq  F value Pr(>F)    
logb(body.volume)     1 174.67  174.67 4353.391 <2e-16 ***
sp                    2   0.03    0.02    0.381  0.684    
logb(body.volume):sp  2   0.05    0.03    0.636  0.532    
Residuals            78   3.13    0.04                    
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
> 
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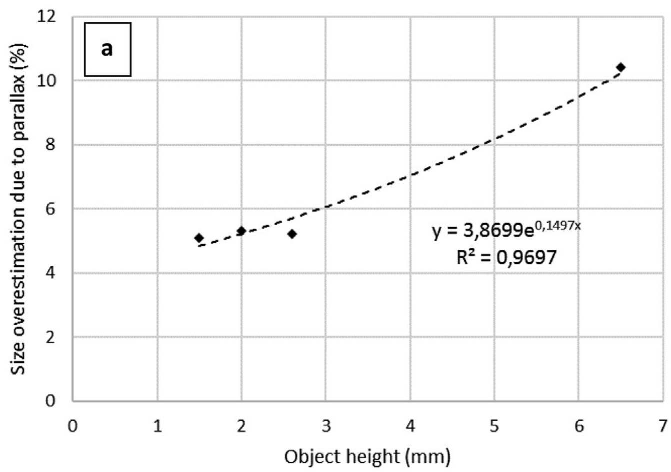
Fig. A2. Summary statistics of ANCOVA testing for the differences in slope between the three diplopod species for lenght-biomass (a), width-biomass (b) and volume-biomass relationships (c).



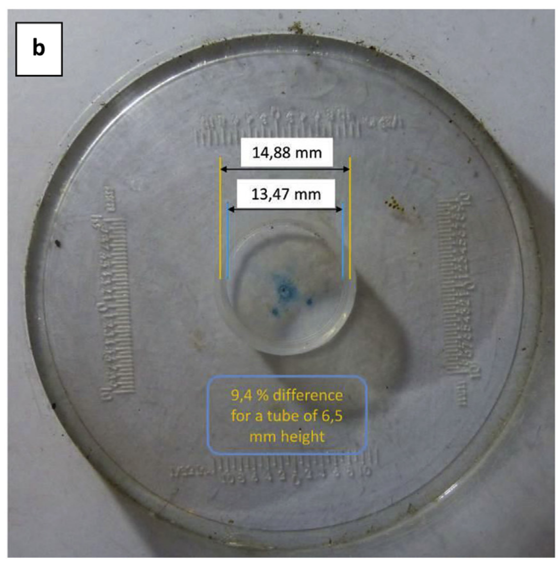


Fig. A3. Protocol for correcting parallax error. Tubes of different height were measured using the same configuration as for animal measurement (A3.a) and parallax was estimated as the % of difference between the tube diameter at the bottom of the box and the tube diameter on its top. A calibration curve was then established for predicting parallax error for each sample of the data set (A3.b). We considered that width was measured at mid-height of animal and that animal are as high as width so parallax error for width data were corrected using ½ of width value. Then we consider that length was measured at the top of the animal so parallax error for length was estimated using (corrected) width values.

Appendix B. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ejsobi.2017.09.006.

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