Soil macrofaunal biodiversity in Amazonian pastures: Matching sampling with patterns

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Abstract

Soil biodiversity varies through space as influenced by habitat features and land-use history. The performance of any sampling strategy highly depends on its relevance with regards to this pattern. We surveyed the soil macrofaunal species richness in the pastures of the Benfica Field Station (Eastern Amazonia, State of Pará, Brazil) and described its variability in 4 independent replicate plots. We designed a within-plot sampling scheme that accounted for the soil spatial variation (stratified sampling). Replicated pasture plots had different species richness (49–65) corresponding to a low proportion (40–53%) of the total number of species (123). Pairs of replicated plots showed an outstandingly low number of shared species (28–41% of the species pool). Likewise, different classes of soil thickness, corresponding to a Ferralsol–Cambisol sequence, had different species richness (12–44) and exhibited a very low proportion of shared species (15–29%). The proportion of rare species, i.e. singletons, ranged from 40–51% of the total species richness depending on the plot considered. We used the abundance-based coverage estimator of species richness (ACE) and the Chao shared species estimator that provides a correction based on the relative abundance of rare species. These indices also showed both a high between plots dissimilarity and a substantial within plot variability of species composition. Because of the high proportion of rare species, the rarefaction curves failed to reach any asymptote in all replicated plots. Bootstrap resampling showed that less than 5 samples per stratum (class of soil thickness) provided inconsistent species richness values. We simulated the efficiency of sampling strategies that included our 4 replicate plots and the 3 classes of soil thickness but with varying sampling effort within each stratum. The results indicated that a fairly large (74%) proportion of species would be recorded if strata were sampled using 5 sampling units (hence 15 samples per plot for a total of $4 \times 15 = 60$ samples). This study showed the need for adequate plot replication in soil macrofaunal biodiversity studies. Also, the main relevant factors of within-replicate plot spatial heterogeneity (e.g. soil, vegetation) should be accounted for through stratified sampling. The results showed that there is no way of reducing the local sampling effort below a certain level (here, 5 sampling units per stratum).

1. Introduction

Below ground organisms are critical in determining the functioning of agro-ecosystems. Soil biota substantially regulate different processes such as decomposition or nutrient mineralization/cycling. Hence, they partly determine plant growth and sustain the long-term productivity (Lavelle, 1997; Wardle et al., 1999; Wolters, 2001). For this reason soil organisms are increasingly considered as a resource to be managed and protected. Soil macrofauna (animals with body length >2 mm) are dramatically affected by cultural practices and various authors have discussed the utility of managing their populations to

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improve the sustainability of soil fertility especially in countries or regions where farmers have limited access to inputs (Matson et al., 1997).

The management of soil biota populations in agro-ecosystems and more generally the assessment of their density, biomass and biodiversity first imply that an adequate sampling protocol is established. In the tropics, soil macrofauna is often sampled following the recommendations of the Tropical Soil Biology and Fertility (TSBF) programme (Anderson and Ingram, 1993). The method is a standardised sampling procedure providing reliable estimation of the mean density and biomass of 15 broad taxonomic groups of soil macrofauna. Numerous studies are based on this protocol (see e.g. Lavelle and Pashanasi, 1989; Brown et al., 2004; Decaëns et al., 2004; Jiménez and Decaëns, 2004; Rossi and Blanchart, 2005). However, in recent years species-level studies have become more frequent due to an increasing interest in biodiversity and its conservation (Thomas et al., 2004; Mathieu et al., 2005; Nahmani et al., 2006). As a consequence the goal of sampling has changed from providing reliable high taxonomic level information to estimating the number of species and their abundance.

However, there is little information on the ability of the TSBF protocol to provide accurate estimates of species richness or diversity. The first obvious question is the number of plots that should be sampled. Anderson and Ingram (1993) only recommended that a transect be randomly positioned within the sampled plots. However, as random sampling implies that the object under study be homogeneous, the accuracy of such a protocol will depend on the degree of heterogeneity of soil biodiversity. When heterogeneity is high, stratified sampling could be useful. The stratification factors may be major pedological, topographical or vegetational features of the survey plot. Besides, the accuracy of biodiversity estimates is known to vary drastically according to the sampling effort as measured by the number of sampling units or the number of individuals collected (Gotelli and Colwell, 2001). There is only a limited amount of information about these relationships for soil macrofauna but it is a priori necessary to collect more than one replicated sampling unit in each locality (e.g. sample by transect). Although the present study does not address that problem, it must be mentioned that the timing of the study is a major impediment to biodiversity estimates (Rossi and Blanchart, 2005).

Because the patterns of variation in soil biodiversity have obvious consequences on the accuracy of sampling strategies we first characterized the spatial heterogeneity of species richness. We then investigated the effect of plot replication upon the accuracy of species richness estimates. We also quantified the gain of precision obtained when sampling was stratified according to major soil profile features. Finally we determined the performance of different sampling regimes (i.e. number of sampling units or number of individual specimens collected in each locality) in assessing the soil macrofauna biodiversity. This work is based on a large data set collected in the pastures of the Benfica Field Station, Eastern Amazonia (Mathieu et al., 2004).

2. Material and methods

2.1. Site

The study was undertaken in a 7-year-old community of smallholders, Benfica ($5°16'$ S and $49°50'$ E), near Marabá, State of Pará, Brazil. We investigated different pasture plots planted with the perennial introduced African grass *Brachiaria brizantha* cv. Marandu. These plots are primarily used for cattle ranching. The climate is tropical humid with a rainy season generally starting in November/December and ending in May/June. The annual rainfall reaches 1800 mm and the average temperature is 26 °C. The region where the survey was carried out comprised fragmented landscapes mainly consisting of a network of 50-m-high hillocks mostly covered by forest and pasture. The investigated pasture plots were 4–6 years old and dominated by the grass *Bracharia bryzantha* cv. Marandu. Soils are clayey with varying thickness of the aggregated, macroporous and permeable horizons, above compact alterites (subsoil). The soil thickness decreases from more than 3 m to less than 1 m from the top to the bottom of the hillocks. The phenomenon corresponds to a Ferralsol–Cambisol transition (ISSS Working Group RB, 1998). Uphill, the Ferralsol thickness allows deep roots growth and vertical water drainage. In the middle of the slope the soil thickness is intermediate and a change in soil colour from strong brown to yellowish brown reveals poorer drainage conditions. Downhill, on steeper slope, the permeable horizons are thin (Cambisols) and the water drainage is consequently lateral and superficial. Such differences in water dynamics (Molicova et al., 1997) have important impacts on soil geochemical functioning (Grimaldi et al., 2004) and vegetation structure (Sabatier et al., 1997).

2.2. Sampling

2.2.1. Background information: tropical soil biology and fertility (TSBF) procedure

Sampling units consist of $25 \text{ cm} \times 25 \text{ cm}$ by 30 cm deep soil monoliths. A minimum of 5 and preferably 10 soil monoliths per plot are recommended (Anderson and Ingram, 1993). Sampling units are located 5 m apart and distributed along a transect which is set at random in the plot (Anderson and Ingram, 1993, p. 16–17).

2.2.2. Pasture site replicates

Four pasture sites of 6 ha on average were investigated at the end of the rainy season in 2002. These pastures were 4–6 years old and had similar land-use history and vegetation cover and were separated by plots corresponding to various

land-uses like rice culture, fallow land of various ages and patches of primary forest.

2.2.3. Within-plot stratification

Within each pasture plot, 3 strata were delineated according to the thickness of the soil. The soil thickness classes were >1.2, 1.2–0.6 and $<0.6\,m$ and corresponded to the Ferralsol–Cambisol transition (ISSS Working Group RB, 1998) defined above. The main difference between our sampling protocol and the standardized TSBF approach is that we split the unique transect recommended in the TSBF method into 3 sections which are placed to run through the main pedological features i.e. 3 different levels of soil thickness.

2.2.4. Sampling in the strata

Soil macrofauna was sampled by means of 5–15 soil monoliths spaced 5 m apart along a transect located in each stratum. The number of samples taken for each stratum was uneven due to technical constraints, however it was kept above the minimum level (i.e. 5) recommended by Anderson and Ingram (1993, p. 17). At each sampling point, a metallic frame ($25 \times 25\,cm^2$) was inserted in the soil and the litter was collected. A trench was then dug to a depth of 30 cm around the $25 \times 25\,cm^2$ area to get a soil monolith. Macroinvertebrates from soil and litter were hand-sorted and preserved in 4% formalin solution. In the laboratory, invertebrates were counted and identified at the species level with the help of different taxonomists.

Sampling was done at the end of the rainy season in 2002, when communities were presumed to be at peak of abundance and biomass (Anderson and Ingram, 1993). Data collected in the soil and the litter layer were combined. Overall we sampled 4 replicated pasture sites, within which each 3 soil depth strata were investigated each time. In each case from 5 (the minimum value according to Anderson and Ingram, 1993) to 12 samples were taken (details of the number of samples per stratum is given in Table 7, first line) which led to a total of 84 soil monoliths.

2.3. Data analyses

2.3.1. Species richness estimates

Bootstrapping (Manly, 1997) was used to estimate the species richness. The method relies on the principle that the frequency distribution of species in a sample is the best indicator of that distribution in the sampling universe. Bootstrapping using various sampling sizes (n) corresponds to simulating the performance of different sampling intensities. This allows constructing species rarefaction curves as a function of the number of sampling units or individuals (Gotelli and Colwell, 2001). We used 500 randomisations for the bootstrap and the bootstrap resampling. In each case sampling with replacement was preferred over sampling without replacement because it leads to a variance among randomizations that is meaningful. We developed a Visual Basic 6 software to do the

computations for the simple Bootstrap whereas rarefaction curves (bootstrap resampling) were performed using the software EstimateS (Colwell, 2005).

2.3.2. Stratified sampling simulations

In order to assess the effect of sampling intensity we simulated various sampling regimes ($n = 1$, 2 or 5 sampling units) in all strata (and thus all plots were considered). Each simulation led to 12 (strata) $\times n$ samples and the observed species were recorded. For each n value, 500 randomisations were done and the mean of the species richness was computed.

2.3.3. Shared species

We compared the different plots or strata by computing the proportion of shared species, i.e. those species simultaneously present in a pair of replicates. A low number of shared species implies a low assemblage similarity; hence, a higher number of replicates would be required. We first computed the absolute number of species shared by site pairs and the percentage of shared species expressed as the ratio of the number of common species to the total number of species in site pairs. Unfortunately, the latter simple indices perform poorly for assemblages that include a substantial fraction of rare species (Colwell and Coddington, 1994; Chao et al., 2005). Therefore we used the ACE (Colwell and Coddington, 1994) and the Chao shared species estimator (Chao et al., 2000) that, respectively augment the observed species richness and the number of shared species by a correction term based on the relative abundance of shared rare species. The computations were performed using the software EstimateS (Colwell, 2005).

2.3.4. Measures of rarity

The species rarity was measured as the absolute and relative frequencies of singletons and doubletons, i.e. species with only 1 or 2 individual(s), respectively. We also report another measure of rarity based on the frequency of species: the number of unique and duplicate species, i.e. species that occur in only 1 or 2 sample(s).

3. Results

3.1. Descriptive statistics

A total of 84 soil monoliths were collected and analysed. The effort (monolith collection and hand-sorting of macrofauna) was ca. 1.5 person hour per sample. It must be noted that this effort was highly variable depending on the density of social insects (termites, ants) that can occur in huge numbers and slow down the hand-sorting process. Overall, this sampling produced 2530 individuals and 123 species. The frequency distributions of species abundance were highly skewed to the right in all plots thus indicating that the assemblages contained mostly species with low abundance (Fig. 1). The most abundant species had
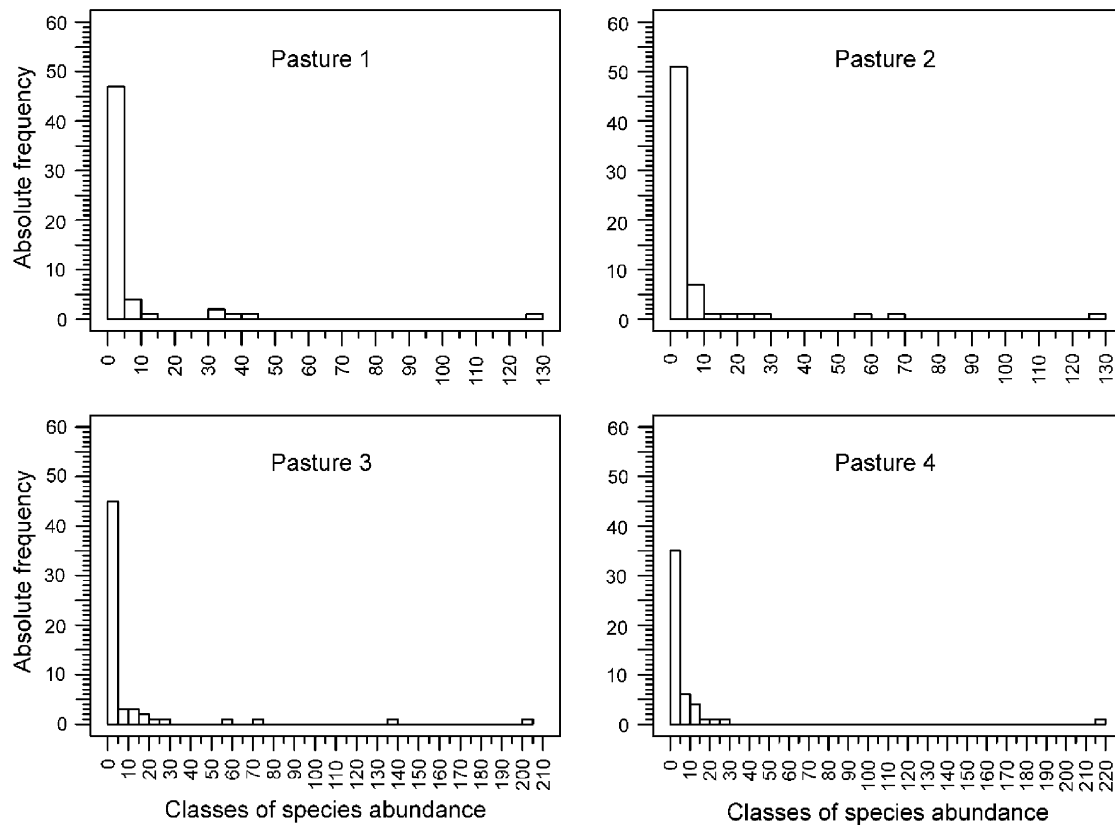
Fig. 1. Frequency distribution of species abundance in 4 replicated pasture plots in Eastern Amazonia. Species abundance is expressed in individuals m^{-2}.

Table 1
Descriptive statistics for soil macrofauna species density in 4 replicated pasture plots in Eastern Amazonia

Replicates	Minimum	First quartile	Median	Mean	Third quartile	Maximum	Standard deviation
Pasture 1	0.7	0.7	1.3	6.7	2.7	128	18.9
Pasture 2	0.6	0.6	1.3	6.9	3.8	125.4	18.8
Pasture 3	0.8	0.8	1.6	11.6	3.6	204.8	33.5
Pasture 4	1.1	1.1	1.1	8.9	6.4	219.7	31.3

Species abundance is given in individuals m^{-2}. The number of samples collected in each plot is indicated in Table 2, first row.

densities ranging from 125.4 to 219.7 individuals m^{-2} according to the plot considered (Table 1). The density of the least abundant species ranged from 0.6 to 1.1 individuals m^{-2} (Table 1). The mean density was somewhat different according to the plot with 11.6 and 6.7 individuals m^{-2} in plot 3 and 1, respectively (Table 1). The standard deviation of soil macrofauna density was variable as well (Table 1). The median and the quartiles reflected the shape of the strongly skewed frequency distribution (Table 1, Fig. 1).

3.2. Species rarity

The proportion of singletons was very high and ranged from 40.4 to more than 50% of the species (Table 2). The proportion of doubletons remained lower (6.1–22%; Table 2). In addition, the proportion of unique species was

outstandingly high ranging from 54.4% to 62.7% (Table 2) which indicated that apart from the singletons, numerous species encountered more than once were only observed in a single sample. This reflected the species spatial aggregation. Not surprisingly the pattern of distribution of the individuals between species (Fig. 2) revealed a low equitability and assemblages mainly dominated by a few species with high densities. Again, the distribution of individuals between species highlighted the importance of rare species.

3.3. Assemblage richness and shared species

3.3.1. Replicate plots scale
Comparing the data collected in 4 different pastures revealed huge differences in terms of species composition (Table 3). The total number of species varied between plots

Table 2
Measures or rarity of the soil macrofauna species in 4 replicated pasture plots in Eastern Amazonia

	Pasture 1	Pasture 2	Pasture 3	Pasture 4
Number of samples collected	24	25	20	15
Number of individuals collected	571	696	852	411
Observed species richness	57	65	59	49
Singletons	23 (40.4)	31 (47.7)	27 (45.8)	25 (51)
Doubletons	11 (19.3)	6 (9.2)	13 (22)	3 (6.1)
Uniques	31 (54.4)	39 (60)	37 (62.7)	27 (55.1)
Duplicates	14 (24.6)	11 (16.9)	8 (13.6)	9 (18.4)

Singletons and doubletons are species with only 1 or 2 individual(s). Uniques and duplicates are species occurring in only 1 or 2 sample(s). Ratios to the observed species richness are given in parentheses. Note that the number of individuals collected in each plot is not directly comparable since the sampling effort differed amongst replicate plots.
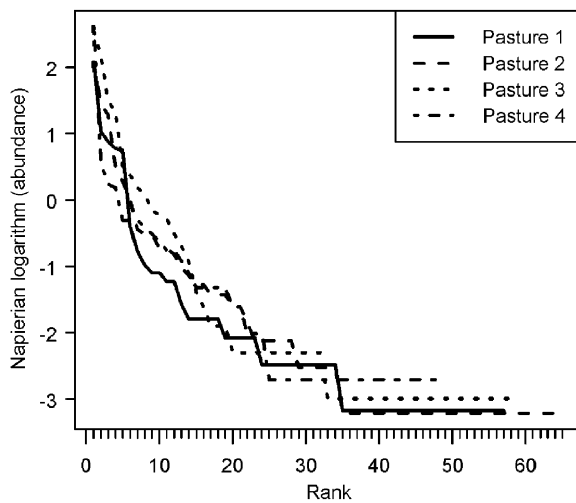

Fig. 2. Rank order abundance plots for species assemblage of soil macrofauna in 4 replicated pasture plots in Eastern Amazonia.

Table 3
Observed species richness and shared species in 4 replicated pasture plots in Eastern Amazonia

	Pasture 1	Pasture 2	Pasture 3	Pasture 4
Pasture 1	**57 (43.6)**	32 (90)	30 (86)	23 (83)
Pasture 2	35.6	**65 (52.8)**	36 (88)	27 (87)
Pasture 3	34.9	40.9	**59 (48)**	24 (84)
Pasture 4	27.7	31	28.6	**49 (39.8)**

The total number of species is 123 (all replicates pooled). *On the diagonal*: observed species richness and the corresponding ratio to the total species richness (between parentheses). *Above the diagonal*: absolute number of species shared by site pairs and total number of species collected in pairs of sites (between parentheses). *Below the diagonal*: percentage of shared species expressed as the ratio of the number of common species to the total number of species in site pairs.

(49–65) and always corresponded to a limited proportion of the total number of species (123 species, i.e. 39.8–52.8%). Moreover, replicate comparison showed that the absolute number of shared species (Table 3, above diagonal) and the corresponding proportion of the observed species (Table 3, below diagonal) were always low (27.7–40.9%) regardless of the plot considered.

Table 4
Estimated species richness and estimated number of shared species in 4 replicated pasture plots in Eastern Amazonia

	Pasture 1	Pasture 2	Pasture 3	Pasture 4
Pasture 1	**86 (32.4)**	37 (167)	56 (145)	32 (147)
Pasture 2	22.2	**118 (37)**	71 (162)	91 (121)
Pasture 3	38.6	43.8	**115 (33.6)**	42 (166)
Pasture 4	21.8	75.2	25.3	**93 (27.9)**

The ACE estimation total number of species is 176 (all sites pooled). *On the diagonal*: ACE estimates of species richness and the corresponding ratio to the ACE estimation total number of species (between parentheses). *Above the diagonal*: Chao estimates of the shared species by site pairs and ACE estimate of the total number of species in pairs of sites (between parentheses). *Below the diagonal*: percentage of shared species expressed as the ratio of the number of common species to the total number of species in site pairs.

The ACE estimation of the species richness and the Chao estimate of shared species are reported in Table 4. The overall species richness estimated by ACE was 176, a value noticeably larger than the observed richness (123). Likewise the Chao estimates of shared species were conspicuously higher than the observed values (Tables 3 and 4). However, when we examined the percentage of shared species we still had low similarity between replicates except in one case; the pair of sites 2 and 4 for which the proportion of shared species reached the value of 75.2% (for a total of 121 species) (Table 4). This high similarity was due to a somewhat high value of the Chao shared species estimator (91). With this exception, our results showed that using the advanced biodiversity indices estimations, although providing improved estimates, did not change the interpretation of the raw data (recorded directly from the field: Table 3) that is replicate pasture plots were very heterogeneous in terms of species composition.

3.3.2. Influence of soil cover organization

Examining the species richness in 3 main soil thickness classes within each replicated plot revealed the substantial effect of lateral variation of soil cover from uphill to

Table 5
Observed species richness and shared species according to the soil thickness classes (High: >1.2 m, Medium: 1.2–0.6 m, Low: <0.6 m) in 4 replicated pasture plots in Eastern Amazonia

	Soil thickness		
	High	Medium	Low
Pasture 1			
High	**41 (71.9)**	7 (46)	11 (53)
Medium	15.2	**12 (21.1)**	5 (30)
Low	20.8	16.7	**23 (40.4)**
Pasture 2			
High	**44 (67.7)**	12 (55)	16 (55)
Medium	21.8	**23 (35.4)**	9 (41)
Low	29.1	22	**27 (41.5)**
Pasture 3			
High	**44 (74.6)**	9 (52)	10 (52)
Medium	17.3	**17 (28.8)**	7 (28)
Low	19.2	25	**18 (30.5)**
Pasture 4			
High	**17 (34.7)**	6 (35)	6 (34)
Medium	17.1	**24 (49)**	6 (41)
Low	17.6	14.6	**23 (46.9)**

On the diagonal: observed species richness and the corresponding ratio to the total species richness (between parentheses). The observed total number of species in each plot is given in the third row of Table 2. *Above the diagonal*: absolute number of species shared by site pairs and total number of species collected in pairs of sites (between parentheses). *Below the diagonal*: percentage of shared species expressed as the ratio of the number of common species to the total number of species in site pairs.

Table 6
Estimated species richness and estimated number of shared species according to the soil thickness classes (High: >1.2 m, Medium: 1.2–0.6 m, Low: <0.6 m) in 4 replicated pasture plots in Eastern Amazonia

	Soil thickness		
	High	Medium	Low
Pasture 1			
High	**71 (87.4)**	12 (78)	13 (89)
Medium	15.4	**19 (23.4)**	5 (45)
Low	14.6	11.1	**31 (38.2)**
Pasture 2			
High	**81 (68.3)**	18 (121)	20 (108)
Medium	14.9	**58 (48.9)**	11 (94)
Low	18.5	11.7	**47 (39.6)**
Pasture 3			
High	**88 (76.6)**	17 (105)	12 (97)
Medium	16.2	**34 (29.6)**	11 (44)
Low	12.4	25	**21 (18.3)**
Pasture 4			
High	**28 (29.9)**	8 (61)	6 (57)
Medium	13.1	**41 (43.8)**	7 (69)
Low	10.5	10.1	**35 (37.4)**

The ACE estimation total number of species for plots 1, 2, 3 and 4 is, respectively 81, 119, 115, and 94 (all strata pooled site by site). *On the diagonal*: ACE estimates of species richness and the corresponding ratio to the ACE estimates of plot species richness (between parentheses). *Above the diagonal*: Chao estimates of the shared species by site pairs and ACE estimate of the total number of species in pairs of sites (between parentheses). *Below the diagonal*: percentage of shared species expressed as the ratio of the number of common species to the total number of species in site pairs.

downhill upon species pattern (Table 5). The soil thickness strongly affected soil macrofauna species richness as e.g. 41, 12 and 23 species were recorded in plot 1 for high, medium and low depth classes, respectively (Table 5). The different strata represented a variable proportion of the plot species richness (Table 5, diagonals). This proportion ranged from 21.1% to 74.6%. The number of shared species as well as the corresponding proportion of the total number of species remained low in all plots and ranged from 14.6% to 29.1% (Table 5). The ACE estimation of the total number of species for plots 1, 2, 3 and 4 was, respectively 81, 119, 115, and 94 (all strata pooled, Table 6). ACE estimates of species richness were considerably higher than the corresponding observed values (Tables 5 and 6) conveying the importance of rare species in the richness estimation process. However, while the absolute values were larger, the corresponding proportions remained almost unchanged (Tables 5 and 6) thus the ACE estimation confirmed that each soil stratum hosted only a small subset of the total species pool. ACE and Chao analyses led to very low proportions of shared species between soil strata ranging from 10.1% to 25% (Table 6). As reported in the case of the plot-scale analyses, our results showed that using the advanced biodiversity indices produced valuable corrected values but did not change the interpretation of the data.

3.4. Effect of sampling regime

3.4.1. Replicate plots scale

We simulated various sampling intensities for each plot, separately. We report rarefaction curves with sampling with replacement in Fig. 3(A). The curves appeared very close to one another and they failed to reach any clear asymptote. Therefore comparing these plots in terms of species richness was done by plotting richness as a function of the accumulated number of individuals instead of the accumulated number of samples (Gotelli and Colwell, 2001). The resulting rarefaction curves (Fig. 3(B)) showed that the pasture plot 3 differed from the other replicates by a lower richness. This plot displayed a much higher macrofauna density (Table 1) but not a higher observed species richness (Table 3, diagonal) therefore the rate of richness increase is lower when it is expressed as the accumulated individuals instead of accumulated samples.

3.4.2. Soil strata

Table 7 shows the average species richness as estimated by bootstrap in each stratum. We show estimations based on 1, 2 and 5 samples. The latter sampling intensity within each stratum (hence a total of 15 sampling units)
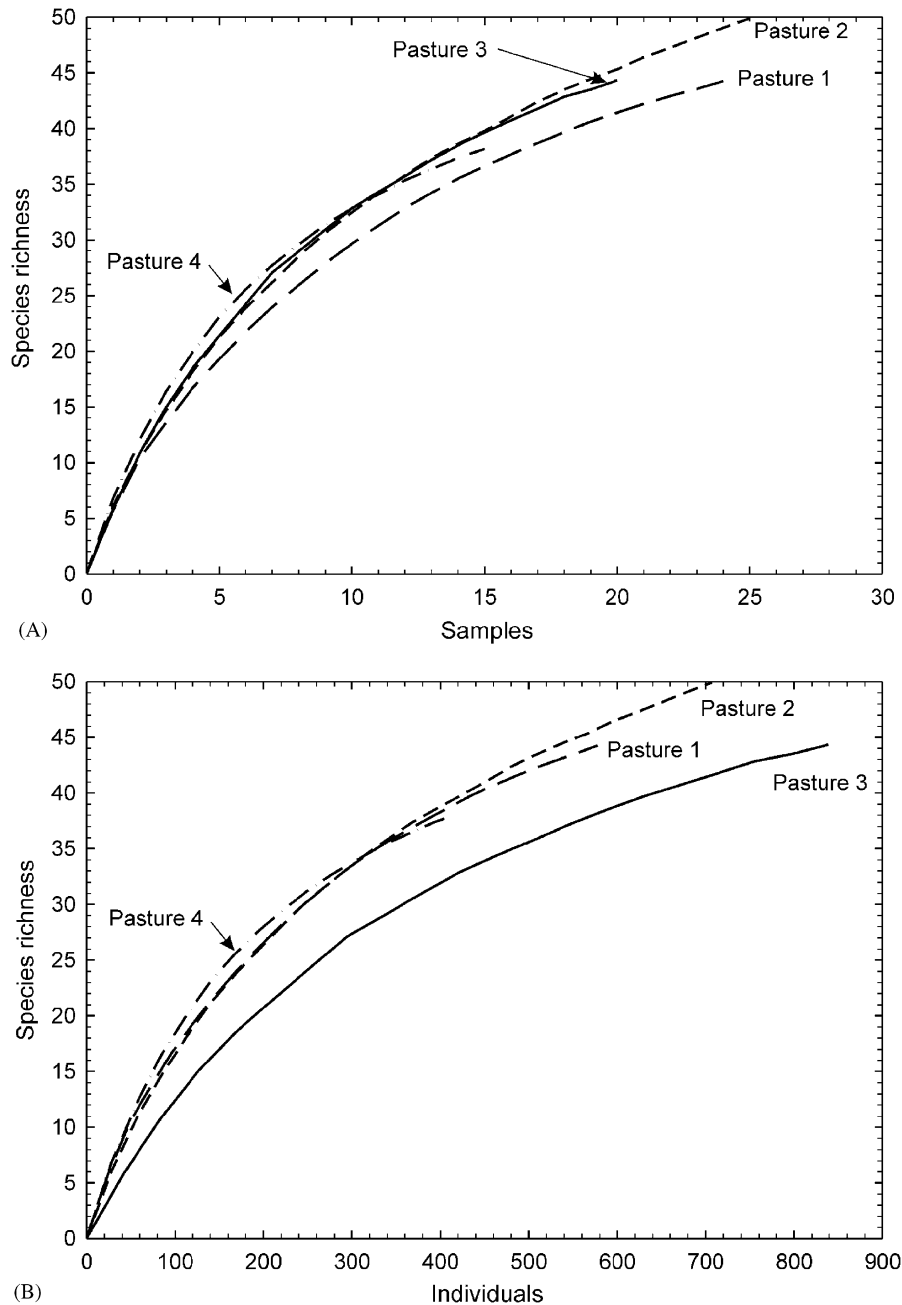
Fig. 3. Rarefaction curves for species richness of soil macrofauna in 4 replicated pasture plots in Eastern Amazonia. (A) Species richness versus accumulated samples (B) Species richness versus accumulated individuals. In each case 500 randomizations were performed (sampling with replacement).

corresponded to a typical TSBF transect (Anderson and Ingram, 1993). It can be seen that sampling using only one sampling unit invariably performed poorly with species richness estimates ranging from 15% to 34.3% of the overall richness. When two replicates were used the estimates improved to attain the values of 25.9–53.5%. Finally we obtained estimations ranging from 49.3% to 80% of the total richness for 5 replicates in each stratum (i.e. a total of 15 samples by replicate plot). Using all the available individual samples (i.e. bootstrapping with $n = N$) led to estimations ranging from 72.2% to 81.7% of the total species richness (Table 7).

3.4.3. Stratified sampling simulations

Table 8 gives the results of our stratified sampling simulations. We indicate the overall estimate of species richness for a simulated sampling scheme that included all the plots and strata but corresponded to different sampling intensities. Recall that the observed total species richness across the pastures plots was 123 species. It can be seen from Table 8 that the accuracy of the estimates increased regularly from 43.2 species (35.1% of the total) for 1 sample in each stratum to 90.6 species (73.7% of the total) when all strata were sampled using 5 samples. The latter sampling intensity corresponded to 15 samples per plot

Table 7

Observed and bootstrapped species richness of soil macrofauna in 4 replicated pasture plots and 3 classes of soil thickness (High: >1.2 m, Medium: 1.2–0.6 m, Low: <0.6 m)

	Pasture 1			Pasture 2			Pasture 3			Pasture 4		
	High	Medium	Low	High	Medium	Low	High	Medium	Low	High	Medium	Low
N	9	5	10	12	5	8	7	5	8	5	5	5
S	41	12	23	44	23	27	44	17	18	17	24	23
$S_{boot}(n=1)$	8.2 (20)	3.9 (32.5)	4.2 (18.3)	6.6 (15)	6.6 (28.7)	4.6 (17)	9.6 (21.8)	4 (23.5)	4.1 (22.8)	5.6 (32.9)	6.9 (28.8)	7.9 (34.3)
$S_{boot}(n=2)$	13.5 (32.9)	6 (50)	7 (30.4)	11.4 (25.9)	10.5 (45.7)	8.6 (31.9)	16.5 (37.5)	6.8 (40)	7.1 (39.4)	8.9 (52.4)	11.3 (47.1)	12.3 (53.5)
$S_{boot}(n=5)$	23.7 (57.8)	9.2 (76.7)	12.8 (55.7)	21.7 (49.3)	17.2 (74.8)	15.9 (58.9)	28.7 (65.2)	11.8 (69.4)	12.3 (68.3)	13.5 (79.4)	18.2 (75.8)	18.4 (80)
$S_{boot}(n=N)$	31 (75.6)	—	17.6 (76.5)	33 (75)	—	19.5 (72.2)	33.3 (75.7)	—	14.7 (81.7)	—	—	—

N: total number sampling units, S: observed richness. $S_{boot}(n)$: bootstrap estimate of the richness for sampling effort of n samples. The corresponding ratio to the observed species richness is given between parentheses. When $n = N$ the results correspond to the classical bootstrap where the simulated sampling effort equals the actual number of sampling units (sampling with replacement). In each case 500 samples were randomly taken.

Table 8

Simulations of different sampling intensities in 4 replicated pasture plots and 3 classes of soil thickness (High: >1.2 m, Medium: 1.2–0.6 m, Low: <0.6 m): effects on soil macrofauna species richness estimate

Number of strata	n	Absolute n	Species richness		% of the total Species richness
			Mean	Standard Deviation	
12	1	12	43.2	6.3	35.1
12	2	24	62.5	6.4	50.8
12	3	36	74.7	6.5	60.7
12	4	48	83.8	6	68.1
12	5	60	90.6	5.7	73.7

Three classes of soil permeable horizon thickness (strata) were investigated in each of the 4 plots. n: number of sampling units per strata. Absolute n: total number of samples (all strata pooled) = $12 \times n$. Mean and standard deviation of the species richness are estimated over 500 randomisations. The % of the total species richness is the ratio of the average estimated values to the overall species richness i.e. 123.

which is typically what Anderson and Ingram (1993) recommend in their handbook of methods. However, here the sampling effort is split into 3 parts and is distributed over 3 strata.

4. Discussion

4.1. Spatial variability of soil macrofaunal biodiversity

Because pastures result from forest clearing and subsequent cultures, communities may diverge because (i) the species pool that withstood forest clearing differed among replicates and thus led to differences in the resulting community structure; (ii) dispersal constraints; (iii) colonisation may be still in progress hence leading to apparent (but transient) differences, and (iv) a synergy of the previous hypotheses. The first hypothesis remains to be tested and necessitates new fieldwork. Such data would be very interesting in that it would be possible to assess the horizontal variability in species biodiversity at the origin of

the colonisation process. Quantitative data are lacking but it is known that some species can survive the primary forest clearance and burning, and pasture establishment. Different species were recorded in a forest plot recently burnt by Amerindians in French Guiana (Rossi et al. in prep.). Whether these surviving individuals can successfully found new population remains to be investigated but there are cases where earthworm species actually survived very intensive crop systems in temperate agroecosystems. The second hypothesis implies the dispersal constraints. Our replicates share the same regional context thus the candidate species pool for recolonization is identical. For a given plot, dispersal constraints may filter out some species and determine a pool of colonists (Belyea and Lancaster, 1999). Species life history probably constitutes an important factor although landscape configuration and plot history are also meaningful factors. For example, if only the closest neighbouring habitat patches are actually acting as source of colonizing individuals, the landscape configuration is of prime importance. Furthermore habitat patch size, isolation and shape have a strong influence upon species abundance and biodiversity (Chust et al., 2003; Dauber et al., 2003; Fahrig, 2003) and it may be hypothesized that not only the local landscape composition but also its structure plays an important role (With and King, 1999; Berggren et al., 2001).

Few data are available on soil macrofaunal dispersal ability and strategy although it strongly affects the observed species richness (King and With, 2002). Some species are obviously good dispersers (e.g. some Coleoptera, Diptera, termites, ants) and some earthworm species are efficient dispersers as shown by the rapid advance of exotic species invading various part of the world (Hendrix and Bohlen, 2002) or previously unpopulated areas (e.g. Dutch Polder see Marinissen and van den Bosch, 1992). However, it is very difficult to determine whether habitat quality (i.e. environmental constraints) or dispersal constraints and internal processes constitute the main constraint upon soil recolonisation after forest clearing, subsequent cultures and final pasture settlement.

4.2. Implications for soil macrofauna sampling

Bootstrap resampling showed to what extent a limited number of sampling units per stratum would perform poorly in estimating species richness. In the investigated pastures, fewer than 5 samples per stratum provided inconsistent estimates. This result can be linked to an important spatial variability in species short-range distribution. The studied pastures feature alternated grass tufts separated by bare soil as well as dead trees, burnt trunks and other isolated structures which likely affect soil macrofaunal biodiversity. The rarefaction curves we reported in this study all failed to reach an asymptote. This is often the case for invertebrate and microbial assemblages (Anderson and Ashe, 2000; Novotny and Basset, 2000; Gotelli and Colwell, 2001) particularly in tropical habitats. The main reason for the absence of plateau is the important number of rare species (throughout this study we defined rare species as singleton) ranging from 40% to 50%. Similar values are reported by Novotny and Basset (2000), i.e. 45% on average in a study of insect communities in tropical forest.

Measuring diversity while dealing with rare species is usually carried out by using mathematical estimators that attempt to account for possible sampling bias by considering the numbers of rare species in the samples (Colwell and Coddington, 1994; Anderson and Ashe, 2000). Likewise, estimates of the number of shared species can be improved by accounting for rare species (Chao et al., 2000). Using these methods with our data led to new estimates of both species richness and the number and proportion of shared species. However, it did not change the main interpretations of the results: plots are highly dissimilar and the within plots variability of assemblages is also very high (between strata differences). Therefore land-use replication is the only way to get a correct picture of the biodiversity.

The results of our multi-plots and multi-strata sampling regime simulations (Table 8) indicated that a fairly large (74%) proportion of species would be recorded if all 4 plots were sampled within the 3 strata using only 5 sampling units. This result is important because it shows that the traditional TSBF sampling protocol can be simply improved to reach good estimates of species biodiversity by stratifying the sampling scheme so as to account for the main features of the habitat. Here such a stratification led to 3 strata. If these strata were sampled by 5 samples (which gave deemed correct estimates in the randomisations) it would lead to a total of 15 samples per plot which does not differ much from Anderson and Ingram's (1993) recommendations.

Our results are based on the simultaneous assessment of 4 replicates and there is no way of reducing this number given the very high dissimilarity between plots in terms of assemblage composition. This study examines data collected within fairly homogeneous herbaceous habitats where no sampling stratification could be elaborated based on vegetation. Our stratification rests here on the thickness of the aggregated, macroporous and permeable horizons of the soil, which is neither difficult nor time consuming to assess in the field. Obviously, other stratification criteria such as soil mineralogical or organic constitution could have been preferred although soil-water dynamics is known to be very important to vegetation and hence of potentially equal importance to soil fauna (Sabatier et al., 1997). More generally it is difficult to be prescriptive in the positioning of the transects since the spatial heterogeneity of habitat and species assemblages is likely to be different in crops or forest ecosystems for example, and in other physical environments.

5. Conclusions

To conclude, we suggest that a multi-scale approach should be adopted whenever possible. The most relevant (according to the organisms under study) factors of spatial heterogeneity should be accounted for and our results show how harmful it might be to neglect this point. Finally it appears that there is no way of reducing the local sampling effort below a certain level (here, 5 sampling units per stratum in tropical pastures). This implies that even if replicated plots were surveyed, and stratification was performed, if the local (i.e. within-strata) sampling regime is too low then the sampling campaign would yield highly underestimated species richness. Because this study only report data collected in a tropical grassland system, no general methodological recommendations can be proposed. More empirical evidence is required before we can determine with some precision how many "TSBF samples" would be necessary to reach a good estimation of species or taxonomic richness in a given habitat.

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