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ScienceDirect
Forest Ecology and Management 255 (2008) 558–566
Forest Ecology and Management
www.elsevier.com/locate/foreco

Sample sizes for estimating key ecosystem characteristics in a tropical *terra firme* rainforest

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Received 18 January 2007; received in revised form 30 July 2007; accepted 6 September 2007

Abstract

This study evaluated the sample sizes necessary to estimate several soil and vegetation characteristics within 10% confidence intervals with 95% probability in three *terra firme* tropical rainforest sites. Across all three plots, the most spatially heterogeneous variables were measurements of total standing crop root mass, ground surface litter mass, litter fall, root growth and soil respiration which required, on average, 152, 105, 52, 45 and 28 samples, respectively to estimate mean values within 10% confidence intervals with 95% probability. Leaf area index measurements integrated canopy characteristics over a relatively large spatial area and therefore only required five samples, on average, to achieve the same degree of precision. Measurements of soil temperature, moisture, carbon and nitrogen content in the surface 30 cm soil layer displayed the lowest degree of spatial variation: requiring a maximum of seven samples to estimate mean values within 10% confidence intervals with 95% probability. This study, together with a review of data from similar ecosystems, suggests that standing crop root mass, root growth, litter fall and ground surface litter mass are usually acutely under-sampled, which could impede detection and interpretation of patterns and processes in these potentially important ecosystem characteristics. This information may assist researchers to design effective sampling strategies for field experiments, particularly in tropical forests.

Keywords: Amazon tropical rain forest; Spatial heterogeneity; Coefficient of variation; Sample size; Plant biomass; Root production; Soil respiration

1. Introduction

Terrestrial ecosystems play a key role in the global carbon (C) cycle and climate system (IPCC, 2001). The Amazon rain forest alone contains 70–80 billion tonnes of C in plant biomass, and is responsible for up to 10% of global terrestrial net primary productivity (Houghton et al., 2001). However, accurate measurement of terrestrial C cycle rates and processes in the Amazon is hindered both by the considerable time and labour costs associated with measurements, and the high degree of spatial heterogeneity in many C stocks and fluxes.

In this context, sample size analysis is important both at the experimental design stage to calculate the sample size required to estimate mean values of chosen variables within designated confidence intervals and after data collection to estimate confidence intervals around measurements for a chosen sample size. However, given the high costs associated with even preliminary measurements of some variables (e.g., root standing mass and production) few studies have estimated sample size for most major C stocks and fluxes simultaneously.

The purpose of this analysis, therefore, is to provide sample size data to aid decision-making by researchers designing field experiments, particularly in tropical forests. To do this, sample size was estimated for the following ecosystem characteristics

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0378-1127/$ – see front matter
doi:10.1016/j.foreco.2007.09.026

at three 1 ha plots with contrasting soil and vegetation types in an eastern Amazon rainforest:

(1) Soil moisture, temperature, carbon and nitrogen content in the surface 30 cm soil layer.
(2) Leaf area index (LAI), litter fall, ground surface litter, root standing crop and root growth.
(3) Soil respiration.

We then placed these site-specific results into their regional context with a literature review of sample size estimates for the above ecosystem characteristics from other studies in old-growth *terra firme* Amazonian rainforest.

2. Materials and methods

2.1. Study site

The experimental site is located in the Caxiuanã National Forest, Pará State, northeastern Brazil (1°43′3.5″S, 51°27′36″W). The predominant vegetation of the area is of a lowland *terra firme* rainforest with no clear signs of past anthropogenic disturbance. The site experiences high annual rainfall (~2270 mm) and a pronounced dry season (Fisher et al., 2005). The most widespread soil type is a highly weathered yellow Oxisol (US Department of Agriculture soil taxonomy) which exhibits substantial spatial variation in the relative proportion of sand and clay, at all soil depths (Ruivo and Cunha, 2003). There are also areas of relatively fertile soil, called anthropogenic dark earths (ADE) or *Terra Preta do Indio*, which mark locations that were intensively managed by indigenous populations of pre-Columban inhabitants (Ruivo and Cunha, 2003; Lehmann et al., 2003). To represent existing variation in soil type at the site, 1 ha plots were established (see Table 1 for additional plot details) on a well-drained sandy Oxisol (Sand plot), a clay-rich Oxisol (Clay plot), and an ADE (Fertile plot). The plots were selected on the basis that they appeared to be relatively internally homogenous, and samples were only collected over 10 m from the perimeter of each plot to minimize edge effects from surrounding soil and vegetation types (Fig. 1). All measurements were made along a regularly spaced grid at 20 m intervals within each plot, with the exception of rhizotron root growth, which was recorded every 30 m (Fig. 1).

Table 1
Plot mean ± S.D. of key plot vegetation and soil features

	Sand	Clay	Fertile
Vegetation			
Tree density (stems ha^{-1})	434	419	544
Stem basal area (m^2 ha^{-1})	24	25	37
Soil			
Sand content (%)	76 ± 4	38 ± 7	53 ± 5
Silt content (%)	8 ± 2	14 ± 2	23 ± 2
Clay content (%)	16 ± 3	48 ± 9	23 ± 4
Ca^{2+}	56 ± 10	60 ± 10	1925 ± 457
Mg^{2+}	34 ± 15	33 ± 12	260 ± 75
P	3 ± 2	2 ± 2	29 ± 11

Tree number and basal area represent all individuals over 10 cm diameter at breast height, measured in January 2005. Soil data are adapted from Ruivo and Cunha (2003); values are a mean of four replicate measurements from the surface 30 cm soil layer on each plot.

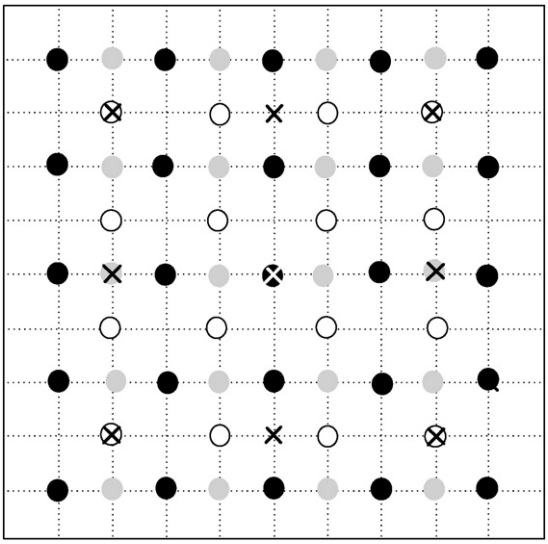

Fig. 1. Spatial sampling strategy within each plot in this study. Solid line: plot perimeter (each side is 100 m); dashed line: 10 m grid within the plot; closed black circles: soil respiration, moisture, temperature, and LAI; open black circles: ingrowth cores; grey circles: litter fall; crosses: rhizotrons, root standing crop, ground surface litter mass, soil C and N content.

2.2. Equipment and measurements

Soil moisture (CS616 probe, Campbell Scientific, U.K.) and soil temperature (Testo 926 probe, Testo Ltd., U.K.) were recorded to a soil depth of 30 cm, in June 2005. Soil samples were taken from the surface 30 cm soil layer of the Sand plot in November 2004, and soil organic C and nitrogen (N) content was determined with a Mass Spectrometer by the Centro de Energia Nuclear na Agricultura, University of São Paulo, Brazil.

Images of the canopy on all plots were recorded with a digital camera and fish-eye lens (Nikon Coolpix 900, Nikon Corporation, Japan) in June 2005. Measurements were taken in the late afternoon when direct sunlight was at a minimum. The images were then analyzed (using Hemiview 2.1 SR1, Delta-T Devices Ltd., U.K.) to calculate LAI over the month of measurement (Hale and Edwards, 2002).

Litter fall accumulation over April 2005 was measured on all plots using mesh traps (area = 1 m^2), placed 1 m above the ground surface. Organic litter was also removed from 154 cm^2 areas of the ground surface in June and November 2005. No attempt was made to separate ground surface litter into different fractions because there was no clear distinction between soil organic matter horizons. Collected samples of litter fall and ground surface litter were cleaned of inorganic debris, dried at 70 °C to constant mass and weighed. There is no significant difference in ground surface litter mass measured between dates and so the data were pooled to calculate CV.

To estimate standing crop root mass, soil cores (diameter = 14 cm; depth = 30 cm) were extracted from all plots in June and November 2005 using opposable semi-circular cutting blades. Roots were manually extracted from the soil cores following the method described by Metcalfe et al. (2007) which corrects for underestimates in, particularly fine, root mass. Root vitality could be reliably assessed visually, and so samples were not divided into live and dead classes. Extracted root material was cleaned of inorganic debris, dried at 70 °C to constant mass and weighed. There is no significant difference in standing crop root mass measured between dates and so the data were pooled to calculate CV.

Root production was estimated on all plots in April 2005 using both the ingrowth core (e.g., Steingrobe et al., 2001) and rhizotron (e.g., Sword et al., 1996) methods (for a detailed review and critique of these, and other methods, see Vogt et al., 1998; Hendricks et al., 2006). At the beginning of November 2004, soil cores (diameter = 14 cm; depth = 30 cm) were extracted from locations on each plot using opposable semi-circular cutting blades, the roots were removed by hand and the remaining soil was reinserted into the holes surrounded by plastic mesh bags (mesh aperture diameter = 1 cm). After a 3-month interval the process was repeated, and retrieved root material was cleaned of inorganic debris, dried at 70 °C to constant mass and weighed. Roots were manually extracted from the soil cores following the method described by Metcalfe et al. (2007) which corrects for underestimates in, particularly fine, root mass. Root vitality could be reliably assessed visually, and so samples were not divided into live and dead classes. The amount of root material, which grew into the mesh bags was used to calculate production for each 3-month interval. Thus, root mass production estimated from the ingrowth cores represented growth accumulated over April 2005 and the two preceding months.

Rhizotrons were constructed from frames, supporting vertically orientated transparent plastic sheets (width = 21 cm; length = 30 cm). Rhizotrons were installed in August 2004 and measurement began in November 2004. Incremental root length extension, as a percentage of existing length, was recorded every 15 days by tracing over roots visible at the transparent plastic face with a permanent marker. This recording methodology may underestimate the length of very fine roots, in comparison to minirhizotrons that use digital imaging, but is less susceptible to breakage and technical faults. This study presents data on relative root length extension 9 months after rhizotron installation, by which time root dynamics on all plots had stabilized (Fig. 2). This period for equilibration is similar to that reported by Hendricks et al. (2006), though other studies recommend allowing for a longer period of equilibration (e.g., Burke and Raynal, 1994; Joslin and Wolfe, 1999; Wells et al., 2002).

Soil respiration was measured on all plots in June 2005 with a closed dynamic infrared gas analyzer (EGM-4 and SRC-1 chamber, PP Systems, U.K.). Plastic collars were inserted 2 cm into the soil at each measurement location 6 months prior to the initiation of respiration measurements. Collar insertion may have caused some degree of disturbance to surface soil and roots but was necessary to ensure a good seal between the IRGA chamber and soil. Soil respiration was calculated from the change in carbon dioxide (CO_2) concentration over time within the IRGA chamber (Blanke, 1996).

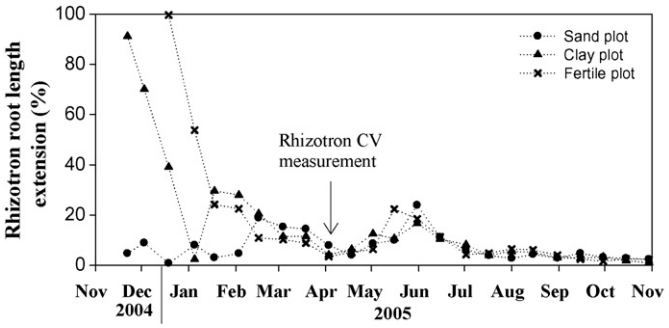

Fig. 2. Relative root length extension for all plots, over 1 year. Measurements of CV and sample size were based upon extension in April 2005, allowing sufficient time for equilibration after the initial disturbance of rhizotron installation. We attribute the later increase in growth (peaking around June 2005), synchronous across all plots, to a real seasonal pattern rather than a disturbance effect.

2.3. Sample size analysis

The equation of Hammond and McCullagh (1978) was used to estimate sample size (SS) for a given confidence interval and probability level:

$$\mathrm{SS} = \frac{t_\alpha^2 \, \mathrm{CV}^2}{D^2} \quad (1)$$

where t_α is the Student's t statistic at a chosen α probability level (0.05 in this study, t for a given α varies with dataset degrees of freedom), CV the sample coefficient of variation (standard deviation of the sample as a percentage of the mean value), and D is the specified confidence interval (10 in this study). Confidence interval specifies the estimated range of values, expressed as a percentage of the estimate of the mean, which is likely to contain the true mean value. Probability level specifies the number of occasions $(1 - \alpha)$ expressed as a percentage that the true mean value would fall within the confidence interval if the measurement were repeated a large number of times.

3. Results and discussion

Estimates of CV provided in this study are, to an extent, specific to the methodology and equipment used. However, given that the methods and equipment used in this study are relatively widespread, CV and sample size estimates provided should still be applicable in a wide range of field studies. Relatively few studies directly present CV values of measured parameters. For purposes of comparison, we derived CV from some studies in the literature indirectly, where necessary, using cited values of sample sizes, means, standard errors, standard deviations, and confidence intervals.

Table 2
Summary of mean ± S.E. CV and sample size calculated for different Amazonian *terra firme* rainforest characteristics from this study, and from other estimates available in the literature

	CV	Actual sample size	Ideal sample size[a]	*n*
Leaf area index	13 ± 1	16 ± 1	9 ± 1	43
Litter fall	40 ± 5	15 ± 2	68 ± 15	22
Ground litter	43 ± 6	15 ± 2	71 ± 14	8
Soil respiration	26 ± 3	24 ± 4	27 ± 6	21
Soil C content	21 ± 6	7 ± 1	30 ± 14	9
Soil N content	23 ± 6	7 ± 2	33 ± 16	7
Soil temperature	1 ± 0.3	19 ± 3	1 ± 0.1	6
Soil moisture	9 ± 1	20 ± 3	2 ± 1	5
Standing crop roots (>2 mm)	54 ± 12	8 ± 2	168 ± 69	10
Standing crop roots (<2 mm)	34 ± 5	13 ± 2	44 ± 11	12
Root growth	55 ± 8	16 ± 2	123 ± 41	14

[a] To estimate the true mean value within 10% confidence intervals with 95% probability. *n* indicates the number of individual plot values from this study and others (presented in Table 3) used to calculate mean ± S.E. values. Minimum ideal sample size is one, though at least two samples are required to calculate standard deviation.

3.1. Spatial variation of soil characteristics

The results of this study are consistent with data from previous studies in the Amazon (Table 3), that all point towards the existence of considerable regional scale spatial heterogeneity within ecosystems that are all usually described as lowland *terra firme* rainforest (Williams et al., 2002; Malhi et al., 2004; Aragão et al., 2005; Sotta et al., 2006). However, in this study soil temperature and moisture displayed very low spatial heterogeneity (CV of 1–12%), while soil C and N content displayed higher (but still low compared to other parameters, see below) CV values of 15 and 9%, respectively (Table 3). Other studies, in comparable ecosystems in the Amazon region, displayed similar trends (Tables 2 and 3). The methods and equipment used in this study, therefore, were able to quantify surface soil C content, N content, temperature and moisture to a high degree of precision relatively easily (Table 3). Other soil characteristics, not measured in this study, may display higher levels of spatial heterogeneity. For example, in a more detailed characterization of soil chemistry at an Amazon forest site (Nepstad et al., 2002), some soil characteristics displayed CV of less than 20% (pH, soil content of K, Mg and SO_4) while soil content of NO_3, NH_4, PO_4 and Ca displayed relatively higher CV values of 60, 28, 50, and 24%, respectively.

Table 3
Literature review of CV and sample size estimated for different Amazonian *terra firme* rainforest characteristics

Reference	Location	Distance between replicates (m)	Replicate plot area (m^2)	CV (%)	Actual sample size	Ideal sample size[a]	Notes
Leaf area index							
Aragão et al. (2005)	Tapajos National	10	2,500	5.3	25	1	
	Forest, Pará, Brazil	10	2,500	5.6	25	1	
		10	2,500	6.6	25	2	
		10	2,500	8.6	25	3	
		10	2,500	8.8	25	3	
		10	2,500	9.3	25	3	
		10	2,500	9.6	25	3	
		10	2,500	10.4	25	4	
		10	2,500	10.6	25	4	
		10	2,500	10.6	25	4	
		10	2,500	10.7	25	4	
		10	2,500	12.1	25	5	
		10	2,500	13.1	25	5	
		10	2,500	13.2	25	6	
		10	2,500	13.3	25	6	
		10	2,500	13.6	25	6	
		10	2,500	14.8	25	7	
		10	2,500	15.9	25	8	
		10	2,500	16.4	25	8	
		10	2,500	20.5	25	13	
		10	2,500	22.5	25	15	

Table 3 (*Continued*)

Reference	Location	Distance between replicates (m)	Replicate plot area (m^2)	CV (%)	Actual sample size	Ideal sample size[a]	Notes
Kalácska et al. (2004)	North Guanacaste, Costa Rica		750	28.4	12	26	
McWilliam et al. (1993)	Reserva Ducke, Amazônia, Brazil		400	8.8	4	5	
Nepstad et al. (2002)	Tapajos National Forest, Pará, Brazil	10	10,000	13.6	86	5	Control, year 1
		10	10,000	15.4	100	7	Drought, year 1
		10	10,000	29.4	86	24	Control, year 2
		10	10,000	32.3	100	29	Drought, year 2
Williams et al. (2002)	Tapajos National Forest, Pará, Brazil	10	2,500	11.0	25	4	
		10	2,500	12.7	25	5	
		10	2,500	13.3	25	6	
		10	2,500	14.0	25	6	
		10	2,500	15.3	25	7	
		10	2,500	15.8	25	8	
		10	2,500	21.9	25	14	
		10	2,500	23.1	25	16	
		10	2,500	26.4	25	21	
		10	2,500	26.8	25	22	
		10	2,500	28.4	25	24	
		10	2,500	28.6	25	24	
		10	2,500	33.3	25	33	
This study	Caxiuanã National Forest, Pará, Brazil	10	10,000	20.0	25	12	Sand plot
Litter fall							
Barlow et al. (2007)	Jari Estate, Pará, Brazil			14.3	5	10	
Luizao (1989)	Near Manaus, Amazônia, Brazil.	<140		15.5	15	8	Plateau, year 3
		<140		23.6	15	18	Valley, year 1
		<140		23.6	15	18	Plateau, year 2
		<140		24.3	15	19	Valley, year 2
		<140		28.5	15	26	Plateau, year 1
		<140		34.3	15	37	Valley, year 3
Martius et al. (2004)	Near Manaus, Amazônia, Brazil.			83.1	20	207	Year 2
				92.7	20	258	Year 1
Nepstad et al. (2002)	Tapajos National Forest, Pará, Brazil		10,000	57.9	25	99	Pre-drought
			10,000	57.9	25	99	Post-drought
			10,000	60.6	25	108	Control
			10,000	69.0	25	140	Control
Salimon et al. (2004)	Near Rio Branco, Acre, Brazil			24.4	5	27	
				34.9	5	56	
				37.3	5	64	
				56.5	5	146	
Selva et al. (2007)	Juruena river, Mato Grosso, Brazil			11.9	4	8	Samples are four different watersheds
Smith et al. (1998)	Curua-Una Forest Reserve, Pará, Brazil		3,000	8.9	3	7	
This study	Caxiuanã National Forest, Pará, Brazil	30	10,000	30.2	20	28	Sand plot
		30	10,000	37.6	20	43	Clay plot
		30	10,000	53.5	20	86	Fertile plot
Ground surface litter							
Martius et al. (2004)	Near Manaus, Amazônia, Brazil		1,600	13.0	20	6	
			1,600	35.2	20	37	
Silver et al. (2000)	Tapajos National Forest, Pará, Brazil		3,600	41.5	15	54	Sand soil,
			3,600	58.7	15	107	Clay soil

Table 3 (*Continued*)

Reference	Location	Distance between replicates (m)	Replicate plot area (m^2)	CV (%)	Actual sample size	Ideal sample size[a]	Notes
Smith et al. (1998)	Curua-Una Forest Reserve, Pará Brazil		3,000	24.1	3	91	
This study	Caxiuanã National Forest,	30	10,000	54.2	9	91	Sand plot
	Pará, Brazil	30	10,000	60.3	9	112	Clay plot
		30	10,000	59.9	9	111	Fertile plot
Soil respiration							
Davidson et al. (2000)	Fazenda Vitoria, Pará, Brazil			30.0	16	28	CV includes data from pasture
Davidson et al. (2004)	Tapajos National Forest,	<40	10,000	18.3	18	11	Drought
	Pará, Brazil	<40	10,000	19.7	18	12	Control
Kursar (1989)	Barro Colorado Island,	5		42.0	90	48	
	Panama	5		43.0	51	50	
Salimon et al. (2004)	Near Rio Branco,			11.5	8	5	
	Acre, Brazil			13.1	8	7	
				16.0	8	10	
				17.1	8	11	
Schwendenmann et al. (2003)	La Selva Biological Station,		300	35.0	32	34	Old alluvium soil
	Costa Rica		300	45.0	32	55	"Residual" soil
Silver et al. (2005)	Tapajos National Forest,		<100	14.6	10	10	Clay
	Pará, Brazil		<100	48.5	10	107	Sand
Sotta et al. (2004)	Cuieiras Reserve, Amazônia, Brazil	10,800		24.5	40	17	
Sotta et al. (2006)	Caxiuanã National Forest,		10,000	6.1	16	2	Sand
	Pará, Brazil		2,500	6.4	8	2	Clay
This study	Caxiuanã National Forest,	20	10,000	33.5	25	33	Sand plot
	Pará, Brazil	20	10,000	26.5	25	21	Clay plot
		20	10,000	31.8	25	30	Fertile plot
Soil carbon content							
Salimon et al. (2004)	Near Rio Branco,			11.2	3 (5)	11	
	Acre, Brazil			20.3	3 (5)	36	
Silver et al. (2000)	Tapajos National Forest,		600	7.0	5 (10)	2	Clay
	Pará, Brazil		600	9.7	5 (10)	4	Loam
			600	10.9	7 (10)	5	Clay
			600	16.3	5 (10)	10	Loam
			600	50.8	5 (10)	98	Sand
			600	51.9	5 (10)	102	Sand
This study	Caxiuanã National Forest, Pará, Brazil	30	10,000	14.5	9 (30)	7	Sand plot
Soil nitrogen content							
Silver et al. (2000)	Tapajos National Forest,		600	12.4	5 (10)	7	Clay
	Pará, Brazil		600	13.2	5 (10)	8	Clay
			600	14.9	5 (10)	11	Loam
			600	29.8	5 (10)	41	Loam
			600	29.8	5 (10)	41	Sand
			600	51.6	5 (10)	121	Sand
This study	Caxiuanã National Forest, Pará, Brazil	30	10,000	8.8	9 (30)	3	Sand plot
Soil temperature							
Sotta et al. (2006)	Caxiuanã National Forest,		5,000	1.8	8 (5)	1	Clay
	Pará, Brazil		10,000	2.2	16 (5)	1	Sand
Kursar (1989)	Barro Colorado Island, Panama			1.6	15 (3)	1	

Table 3 (*Continued*)

Reference	Location	Distance between replicates (m)	Replicate plot area (m^2)	CV (%)	Actual sample size	Ideal sample size[a]	Notes
This study	Caxiuanã National Forest, Pará, Brazil	20	10,000	1.1	25 (30)	1	Sand plot
		20	10,000	0.6	25 (30)	1	Clay plot
		20	10,000	0.7	25 (30)	1	Fertile plot
Soil moisture							
Sotta et al. (2006)	Caxiuanã National Forest, Pará, Brazil		10,000	5.7	16 (30)	1	Sand
			5,000	8.0	8 (30)	3	Clay
This study	Caxiuanã National Forest, Pará, Brazil	20	10,000	7.2	25 (30)	2	Sand plot
		20	10,000	11.1	25 (30)	4	Clay plot
		20	10,000	11.5	25 (30)	4	Fertile plot
Root standing crop mass							
Cavelier (1992)	Barro Colorado Island, Panama		1,200	12.0	10 (<5, 25)	5	
Nepstad et al. (2002)	Tapajos National Forest, Pará, Brazil		10,000	43.4	20 (<2, 10)	57	
			10,000	44.7	20 (<2, 10)	60	
			10,000	34.1	3 (>2, 1200)	99	
			10,000	35.8	3 (>2, 1200)	110	
			10,000	30.4	3 (all, 1200)	79	
			10,000	33.4	3 (all, 1200)	96	
Silver et al. (2005)	Tapajos National Forest, Pará, Brazil	<100		17.2	9 (<2, 10)	11	Sand, year 1
		<100		20.1	15 (<2, 10)	13	Sand, year 2
		<100		22.1	9 (<2, 10)	17	Clay, year 1
		<100		25.0	15 (<2, 10)	20	Clay, year 2
Silver et al. (2000)	Tapajos National Forest, Pará, Brazil		3,600	19.7	7 (<2, 10)	15	Sand
			3,600	25.0	7 (<2, 10)	24	Clay
			3,600	39.9	7 (>2, 10)	60	Clay
			3,600	143.3	7 (>2, 10)	776	Sand
Trumbore et al. (2006)	Fazenda Vitoria, Pará, Brazil	50		46	4 (<2, 10)	68	
This study	Caxiuanã National Forest, Pará, Brazil	30	10,000	61.0	9 (all, 30)	115	Sand plot
		30	10,000	69.9	9 (all, 30)	151	Clay plot
		30	10,000	78.3	9 (all, 30)	189	Fertile plot
		30	10,000	53.1	9 (<5, 30)	87	Sand plot
		30	10,000	49.0	9 (<5, 30)	74	Clay plot
		30	10,000	64.1	9 (<5, 30)	127	Fertile plot
Root growth							
Jordan and Escalante (1980)	San Carlos do Rio Negro, Venezuela			63.9	17 (all, GEC, 40)	125	
Sanford (1990)	San Carlos do Rio Negro, Venezuela	<10		56.6	28 (<2, IGC, 10)	93	
		<10		64.3	28 (<2, IGC, 10)	121	
		<10		67.5	28 (<2, IGC, 10)	133	
Silver et al. (2005)	Tapajos National Forest, Pará, Brazil	<100		32.4	9 (<2, SC, 10)	37	Clay, year 1
		<100		54.3	15 (<2, SC, 10)	92	Clay, year 2
		<100		85.0	9 (<2, SC, 10)	251	Sand, year 1
		<100		140.4	15 (<2, SC, 10)	612	Sand year 2
This study	Caxiuanã National Forest, Pará, Brazil	20	10,000	63.3	16 (all, IGC, 30)	123	Sand plot
		20	10,000	27.6	16 (all, IGC, 30)	24	Clay plot
		20	10,000	33.8	16 (all, IGC, 30)	35	Fertile plot
		20	10,000	39.9	9 (all, R, 30)	56	Sand plot
		20	10,000	20.6	9 (all, R, 30)	15	Clay plot
		20	10,000	22.1	9 (all, R, 30)	17	Fertile plot

[a] To estimate the true value within 10% confidence intervals with 95% probability. All studies included are from apparently primary rainforests which displayed no clear signs of past anthropogenic disturbance. Values in parentheses following actual sample size values for soil and root characteristics indicate sampling depth in cm, root diameter category sampled in mm (only for root standing crop mass and growth) and root growth measurement method (only for root growth). Root growth measurement methods are GEC: growth into excavated cavity; IGC: ingrowth core; SC: sequential core; R: rhizotron. Minimum ideal sample size is one, though at least two samples are required to calculate standard deviation.

3.2. Spatial variation of vegetation characteristics

In comparison to the soil characteristics recorded in this study, measurements of variables relating to vegetation mass and growth were more spatially heterogeneous, and hence required more samples to achieve the same level of precision (Table 3). This result is consistent with data from other studies in the Amazon region (Tables 2 and 3). In particular, standing crop root mass displayed considerable spatial heterogeneity, both in this study (CV of 49–78%, depending upon the plot and root diameter category) and in other comparable ecosystems (Table 3). Similarly, root growth quantified in this study with ingrowth cores and rhizotrons displayed CV of 34–63% and 22–40%, respectively. Litter fall and ground surface litter mass displayed relatively high CV in this study of 30–54% and 54–60%, respectively, and therefore required more samples to derive mean values with a high degree of precision. Leaf area index values integrated measurements over a large spatial area ($>10\ m^2$) compared to litter fall and ground surface litter mass ($<1\ m^2$), and so displayed relatively low CV. The high spatial variability of root and foliage characteristics quantified in this study, combined with the low sample size used by most studies because of substantial resources required to make these measurements, often resulted in a considerable disparity between the actual sample size used and the ''ideal'' sample size required to estimate values within 10% confidence intervals with 95% probability (Tables 2 and 3).

3.3. Spatial variation of soil respiration

In comparison to soil and vegetation characteristics, soil respiration is often more intensively sampled in Amazonian *terra firme* forests (Table 3) and usually displays a lower degree of spatial heterogeneity (CV of 27–34% in this study) than roots and foliage characteristics (Tables 2 and 3). Thus, on average across all of the plots surveyed in this study and others, sample sizes chosen to measure soil respiration are usually closer to the level required to quantify the mean within 10% confidence intervals with 95% probability (Table 2).

4. Conclusion

Results from this study suggest that most sampling effort should be spent quantifying aspects of above- and below-ground biomass and growth such as root biomass and production, litter fall and ground surface litter mass. Attempts to quantify these variables, which do not take enough samples, may find that the large degree of uncertainty surrounding estimates impedes detection and interpretation of existing patterns. This is a key problem because foliage and roots play an important, but poorly understood, role in the structure and function of terrestrial ecosystems. Estimates of CV provided in this study are, to an extent, specific to the methodology and equipment used. Therefore, the sample size estimates provided are most readily applicable to researchers working in similar ecosystems, and with similar methodologies. This work could be extended by recording temporal change in spatial heterogeneity in ecosystem characteristics.

Acknowledgements

This research contributes to the Brazil-led Large Scale Biosphere–Atmosphere Experiment in Amazonia. Work was supported by a Natural Environment Research Council (U.K.) PhD studentship and a Standard Research Grant (NER/A/S/2003/1609), a Royal Society (U.K.) Dudley Stamp Memorial Fund award, and an Elizabeth Sinclair Fund award (School of Geosciences, University of Edinburgh, U.K.). All corresponding authors of articles featured in Table 3 were contacted to confirm details of experimental design. In addition we are grateful to Leonardo Sá and Ima Vieira for their scientific support and collaboration, the Museu Paraense Emilio Goeldi for the use of its field station and laboratory facilities, and Bene and Joca for their committed field work assistance.

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