#1 Response to : Interactive comment on "An analysis of forest biomass sampling strategies across scales" by Hetzer et al.

The paper by Hetzer et al. aims at assessing the effect of sampling strategy for estimating tropical forest aboveground biomass at different spatial scales. While this topic is of importance, it has already been well covered in the litterature. However, the simulated approach developped here have some originality (e.g. the point pattern reconstruction) but, in my opinion, some rather surprising or context-dependent results are due to methodological artefacts as described below. These artefacts are rather difficult to overcome but they should be at the minimum discussed or acknowledged before consideration for publication.

Thank you for your very helpful comments. We prepared a revision of our manuscript that follows your recommendations closely. The main changes are:

a) Consideration of the biome for the biomass sampling

We conducted additional analyses where sampling was carried out only in tropical forest biomes. This could reduce the number of plots required for continental biomass estimates. A further stratification into single biomes did not decrease this number significantly. We added and discuss these findings in the revised manuscript.

b) Renaming the sampling method

We renamed the 'remote sensing sampling' method to 'transect sampling' and considered the implications for remote sensing in the conclusions section (e.g., airplane tracks from LiDAR campaigns).

c) Impact of more variation in high biomass values

The current analysis leads to conservative estimations of necessary sample plots. We agree that the tested biomass maps have limitations due to saturation effects. We conducted an additional analysis with higher variation in high biomass values and discuss the results in the revised manuscript.

We have added our responses to your comments in blue following each comment.

##Major comments

Globally, many statements (see my specific comments) are very basic and already well known in the litterature (e.g. many sentences in the conclusion section). The author C1 should refer more to previous works and concepts, including those developped for temperate forests where a huge research effort on sampling strategy has been done in the past.

Thank you for this helpful comment. We improved our introduction by including a paragraph discussing some state of the art sampling methods for temperate forests where systematic sampling designs were established and evaluated (lines 39-44).

Furthermore, we discuss why these sampling approaches are more difficult to establish in tropical regions. One main challenge is that tropical forests are often more dense than temperate forests (about twice as many trees per km^2 (Crowther et al., 2015)), so measurements are more labor intensive. Another issue is that access to tropical forest regions is often restricted due to topographic, logistic or political reasons (Houghton et al., 2009; Mitchard et al., 2014). This limits a comprehensive sampling as applied for temperate forests. (lines 312-315).

Investigating the effect of spatial scales (local, regional and continental) on sampling strategy is very appealing. However, I am very skeptikal about the use of remote sensing products as reference data. Both Asner and Baccini used passive optical data to extrapolate AGB at large scale and these products are well known to saturate for large AGB (>100-200 t/ha) values leading to a strong underestimation of AGB variability. This effect is well illustrated by the Fig. S2 where the SD of AGB first increase with AGB and then decrease. Theoretically the SD of AGB should continuously increase with the mean AGB (this is why people generally use CV instead of SD for comparison purpose). Thus, the decrease of SD with AGB in Fig. S2 is simply an illustration of the saturation problem so that using these maps, or dowscaling them using such SD pattern, result in a strong underestimation of AGB variation in high biomass areas, which, in my opinion, bring a strong bias in the final results presented here. This is probably the reason why some results are very counter-intuitive, such that plot size does not matter at large scale or that a large number of large plots provide less accurate AGB estimate than a small number of small plots (Lines 157-159).

Thank you for that important remark. It is true that the biomass maps used in our study have their limitations. However, these maps represent currently the only possibility to analyze continent-wide biomass distributions. To overcome specific biomass map artifacts we analyzed different biomass maps (Baccini et al., (2012) and Saatchi et al., (2011); see supplements, table S1).

We agree that the saturation effect has not been considered yet. Therefore we analyzed a second downscaling method, where we assume, as an extreme case, a strongly increasing trend between mean aboveground biomass and its standard deviation (see revised Supplements, section S3). In this case we found that about 121 one-ha plots (instead of 70 one-ha plots derived by the currently used approach) would be necessary for mean biomass estimations of the South America tropical forest. Thus, saturation will increase the number of sampling plots needed. We added (lines 183-186) and discussed (lines 278-283) these results in the revised manuscript.

I had two problems with the simulation of RS sampling. First, RS was simulated as discrete measurements, may be to simulate satellite LiDAR measurements such as those produced by GLASS or GEDI, but there is no justification for that (most satellites produce continuous measurements). This is surprising given that the authors used continuous RS-based maps to validate such RS sampling strategy, which look like a bit skizophrenic.

Thank you for this important comment. After some critical reflection, we decided to call this method ‘transect sampling’ as we focus mainly on the establishment of empirical forest plots with this sampling method (lines 111-120). We emphasized the relevance of this sampling strategy also for remote sensing applications in the conclusions section (lines 316-319), as this transect sampling could be interpreted as proxy for airplane flight tracks from lidar campaigns.

Second, I did not fully understand the methodology. I understood that measurements were simulated at different distance along simulated transects but I did not understand how and if the distance between transects varied or not. I am not even sure that the authors simultaneously simulated several transects as would typically be done by a satelitte. I would suggest to simulate a sampling design similar to the one C2 that was or is adopted by GLASS or GEDI to make this simulation more practical even if this is challenging due to the high resolution of LiDAR footprint (~70 and 20 m) and the abovementioned downscaling problem.

As mentioned above, this transect method sampled plots in North-South transects. Within one transect, the plots had regular distances of 0.5km, 1km or 5 km. The spacing between transects was not regular, but randomly chosen. We revised the method section (lines 112-115) to clarify this approach.

The sampling showed in Fig. 2 illustrates a major problem. Nobody sample at the same time dense humid and dry forests to depict a mean biomass. This is always practically done by forest type using a prior stratification design. The minimum, to have something comparable with the other scales (BCI and Panama) is to focus only on tropical dense humid forests. This may explain why an aggregated sampling design produce such huge errors given that it sample very different forests at the continental scale.

Thank you, this is a good point. In the revised manuscript we combine the biomass map with a biome map (Dinerstein et al., 2017) to distinguish between different vegetation types (see Methods lines 87-90 and revised figure 2). This gives us the possibility to analyze the sampling strategies not only exclusively for tropical forest (covering moist broadleaf, dry broadleaf, conifer and mangrove forest) but also for forests of different biomes separately. After merging these two maps, we found that the number of sampling plots decreases if taking only tropical forest into account (from 102 to 74 for random samples, see revised table 1, "South America (500)"; for aggregated sampling designs see revised figure 6 and revised supplemental figure S4). The additional analysis indicated furthermore, that a stratification into biomes does not lead to a significant reduction of the needed sample plots, since the sum of the plots needed for single biomes (32,200 plots) is similar to the plots needed for an overall forest sampling (36,000 plots). However, this stratification helps to better evaluate sampling effort for each biome. We added the new results (revised figure 4, 5, 6, and S4) and discussed them in the revised manuscript (lines 224-228).

As illustrated in Fig. S3, and by previous studies conducted in BCI, the spatial distribution in AGB do not significantly differs from a random distribution. This explain why, for a given sampled area, using several small or few large plots little impacts your estimates. This should be better explained in the present paper by explicitely mentioning the effect of spatial aggregation on sampling design and by stating that your result would probably not hold at the same scale in many (!) other forests that show strong AGB aggregation patterns (which is the case of most forests).

Thank you for your comment. We will revised the discussion (lines 296-298) by mentioning the effect of spatial aggregation (e.g., Chave et al., (2003); Marvin et al., (2014)).

Note also that the central limit theorem only applies if observations are independents (i.e., in absence of significant spatial structure), such that this theorem is theoretically valid only for the BCI scale in your study.

Thank you for that note, this is a tricky point. It is true that the large scale biomass maps show spatial autocorrelation, but we choose in our sampling strategy "random sampling" the biomass values from random locations of the map. This secures that each of our biomass observation is randomly generated in a way that does not depend on the values of the other biomass observations. We would have an autocorrelation problem with the used biomass map if we apply for example clustered sampling (i.e., select always nearby points with higher probability). In our study we compared the outcome of the central limit theory only with the random sampling, not with the other sampling strategies. For all other sampling strategies the spatial autocorrelation of the underlying biomass map is crucial – therefore we simulated these sampling strategies instead of applying the central limit theorem. We clarified this in the revised manuscript (lines 108-110).

The discussion section may discuss the realism of a random sampling design at the continental scale in Amazonia.

We discuss the realism of sampling in the revised conclusions section. (lines 312-315)

The conclusion section should highlight more the originality of the present work.

We revised the abstract (lines 20-21) and emphasize transferability of the presented methods in the conclusions (lines 318-320).

##Specific comments

Line 27: space lacking: “important(Broich” Done.

Line 29: Are those referenced all provided biomass maps? In this study, we compared the biomass maps of Baccini et al., (2012) and Saatchi et al., (2011), but there are more biomass maps available. We added another reference and placed “e.g.” at the beginning of the references (lines 30-31).

Line 34: Please replace by “so that the local distribution in biomass”. At least remove C3 “local regions”, which is inapropriate. Done.

Lines 34-35: This last sentence is very vague. We deleted this sentence.

Line 45: This is an old reference, what about most recent works such as Baccini and Saatchi maps? We deleted this example and cited a more recent example on uncertainty using field plots (lines 48-49)

Line 49: Assume that plots or biomass are. . .. Done.

Line 52: I don’t see the logic here. First it is obvious that the representativness of a given number of plots is context-dependent and varies with the total area of interest and second the number of plots fall into the recommendation cited line 48 so that it does not illustrate that the number of plots varies according to the sampling design. We agree that this number does not reflect the differences between sampling strategies. We deleted this example.

Figure 1: I would have personally not call the b panel a landscape scale but rather a regional scale. I know that the definition of scale strongly varies in the litterature but I can hardly imagine a landscape of more than 500 km. Thank you. We renamed the term.

Line 73: “determined using allometric relationship” is really vague, unless the methodology is fully described in the Knapp paper. If yes, please add (see Knapp. . .. For details). The methodology is described in the Knapp paper. We added the reference as proposed.

Lines 77-78: The following sentence is useless and confusing (strange to refer to plots for RS maps, we usually use pixels instead): “For this purpose, between 4 and 25 plots from the original map were averaged.” We deleted this sentence and changed the wording from “plot” to “pixels”.

Lines 79-80: This last sentence is useless. Deleted.

Line 81-82: This is not true that the Baccini map mostly derived from LiDAR measurements. The global methodology used was to callibrate GLASS LiDAR footprints with field data and then to calibrate a MODIS product with the calibrated LiDAR measurements. Thus the final product mostly reflect MODIS data, that are very little sensitive to biomass and highly sensitive to cloud cover (e.g. the large area of lower biomass observed on the western coastal area of central Africa, compared to the central basin, C4 is simply due to cloud cover). Thank you for mentioning this important point. We revised this sentence (lines 85-87).

Line 83: Please provide rounded numbers. We replaced this sentence by more information on the maps of South America, Africa and South East Asia (lines 84-80).

Line 86-87: Please replace plot by pixel. Done

Fig. 3 legend: “below the bar ” should be replaced by “above the bar” Deleted.

Lines 143-144: Very obvious and well-known result. We added “ …the expected result…” (line 152)

Lines 154-155: First sentence useless. Deleted.

Lines 180-181: Please reformulate. Deleted.

Lines 230-232: Very obvious. Deleted.

Line 235: If forest types are known a better strategy would be to stratify the sampling by forest types. We revised the sentence (270-272) and discuss stratification as mentioned above.

Lines 255-256: As already shown and discussed by previous works. We added references (lines 291-292).

Line 259: What is a regional scale here? Forest sites comparable to BCI. We changed to term to “local”.

Line 267: For a given sampled area, plot size should not. . ... Done.

Line 270-271: This is what is generally done, remote sensing almost always relies on field data. Please be more explicit. We deleted this sentence and added conclusions for remote sensing sampling (lines 316-319).

#2 Response to : Interactive comment on "An analysis of forest biomass sampling strategies across scales" by Hetzer et al.

This manuscript assesses the ability of different sampling strategies to characterize the overall mean biomass of tropical forests. Although there have been previous studies looking at this, the multi-scale approach and the point-pattern simulation to replicate the spatial clustering of previous studies add novelty, and mean that there is enough new for this to be a useful contribution. There are a number of issues that need to be addressed, primarily through improved discussion.

Thank you for your very helpful comments. We prepared a revision of our manuscript that follows your recommendations closely. The main changes are:

d) Restriction of sampling to forest biomes

Following your suggestion we analyzed the sampling strategies for each biome separately (covering moist broadleaf, dry broadleaf, conifer and mangrove forest). Results are added and discussed.

e) Discussion about the impact of more variation in high biomass values

The current analysis leads to more conservative estimations. We agree that the tested maps have limitations concerning the fine scale variation. Assuming an increased variation in biomass values would lead to a moderate increase in the minimum sample size. We added this aspect in the discussion.

We have added our responses to your comments in blue following each comment.

##Major comments

I agree with the major points raised by Ref1, and won't elaborate on them more here except to say that it would make more sense to me to restrict the allocation of sampling points to a single biome (i.e. moist tropical forests) and areas with forest cover (i.e. above a given threshold in the Baccini map) to more realistically reflect real sampling efforts.

Thank you for this comment. We extended the study by analyzing the sampling strategies across different biomes. Therefore, the biomass map used for continental analyses (Baccini et al., 2012) is merged now with a global biome map (Dinerstein et al., 2017). To exclude rarely vegetated pixels within biomes, we assume a minimum above ground biomass threshold of 25 t/ha (see Methods, lines 87-90). Current results show that there are differences between biomes regarding the sampling effort (see revised figure 4 and figure 5 c-d) We include these additional results in the revised manuscript (lines 174-179 and 224-228).

The analysis of clustered sampling strategies implies a very naïve analysis approach to get an overall mean – just taking an average across plots without considering their configuration. To what extent the performance of clustered plot networks at estimating the overall mean can be improved by analyses accounting of climate and soil covariates and/or spatial autocorrelation to account for this oversampling? I would assume that there would be considerable potential to remove the disruptive effect of non-random sampling, and instead move estimates to a point on the random sampling curve equivalent effective sample size of spatially random plots. Thus existing plot networks, with appropriate analysis, may provide much better estimates of continental mean biomass than implied by this study.

This is a good point. We designed our analysis primarily to explore the effect of spatially clustered vs. random sampling. We therefore agree that existing plot networks, that stratified plots based on additional constraints, may provide better estimates than suggested by a "blind" clustered sampling.

A possible solution to this issue is to expand the pattern reconstruction approach to include additional criteria (ideally those used for selection of the real clustered plot networks, accounting e.g., for climate and soil covariates). If the covariates representing the additional criteria can be mapped in the entire study area, the pattern reconstruction approach can take the additional constraints into account and reject plot configurations that do not agree with these criteria.

However, we believe that such an analysis would be beyond the scope of our current study, but an interesting task for forthcoming studies. We therefore briefly discuss in the discussion section that our clustered sampling strategies do not account for additional criteria that will be used for the design of real plot networks, and propose the above solution for a better assessment of the performance of clustered plot networks (lines 299-304).

It is worth noting that the remote sensing maps used as reference have serious limitations (some pointed out by Ref1). Most importantly, they miss the effect of species composition on biomass, which is driven by wood density and leads to marked spatial patterns in Amazonia. This isn't so much of a problem for this study if the remote sensing reference maps are interpreted as providing realistic examples of large-scale spatial variation in biomass, rather than as real references. I do wonder if this means the large scale reference maps underestimate the extent of fine scale variation due to compositional differences across soil types (for example).

Thank you for your comment. We agree that continental biomass maps have their limitations in terms of fine scale variation. A higher variation of the biomass variation leads to a higher sampling effort, such that our estimated plot number could be interpreted as a conservative estimation. We added an additional analysis where we assume higher variations in high biomass values (see section S3 in the revised Supplements) and discuss this important issue in the revised manuscript (lines 183-186 and 278-283).

Specific comment

The barplots in Figures 3 and 4 could be misinterpreted as giving strong evidence that big plots are best, as they show the that the smaller plot size the more plots are needed. It would be good to also display the change in the area of sampling needed (as is done in the text and table), as that is more relevant to sampling effort.

Thank you. The revised figure 3 includes shapes that represent the total sampling areas.

#3 Response to: Interactive comment on "An analysis of forest biomass sampling strategies across scales" by Hetzer et al.

This manuscript details an interesting and novel approach to estimating forest biomass using a dispersed cluster of forest inventory plots. However, in a way this is a "big data" solution to a problem where the solution does not necessarily consider all the variables necessary to making appropriate and constrained biomass estimates. Forest inventory plots are often chosen based on a wide variety of information including forest, soils, hydrology, topography, climate, etc. and are often not randomly chosen. The classical approach of positioning plots in strategic, representative areas often provides constrained and informed estimates of biomass. That said, now that we have huge amounts of remotely sensed data we can apply "big data" approaches to test the extents and limits of many ecological methodologies. I think that is the advantage of this manuscript that it explores this space and does so in an interesting and informative way.

Thank your helpful comments. We prepared a revision of our manuscript that follows your recommendations. The main changes are:

f) Current forest sampling strategies

We will add paragraphs on current sampling strategies in temperate forests to the introduction. Furthermore, we will discuss the feasibility of adapting those strategies in tropical forests.

We have added our responses to your comments in blue following each comment.

I am honestly torn on whether Amazonia is a perfect test or worst-case scenario fort his methodology. Thinking through this, diversity is incredibly high and there are subset forest types within Amazonia. . ..some that rely heavily on topography/climate such as cloud forests, while there are also dry forests, seasonally flooded forests, and also wet forests. But again, this wide variability may actually be a strength of this approach.

We investigated also the sampling strategies for other continents (see Supplements, Table S1). Results showed that the needed number of randomly sampled plots are higher for the tropical forests of South East Asia (103 plots) and Africa (88 plots) indicating that the tropical forests of Amazonia (74 plots) might be not the worst case, but the best-case-scenario.

I would like to see if compared to temperate forests regions in N. America and Europe as well as boreal areas across the higher latitudes. It would be an interesting comparison to see if those systems diverge wildly from Amazonia.

Thank you for raising this point. Comparisons with temperate regions would be very interesting even though we believe that this would go beyond the scope of this study. For higher latitudes we expect differences in the overall biomass distribution (e.g., due to a different species pool compared to the tropics) but also in the spatial distribution of biomass (e.g., due to forest management). Therefore, there might be also relevant differences in the amount of forest sampling plots needed.

However, forests in temperate regions are mostly monitored with already sophisticated sampling methods (i.e., national forest inventories in North America and Europe). In the revised manuscript, we added text about state of the art sampling methods for temperate forests (lines 39-44) and discuss why these sampling approaches are more difficult to establish in tropical regions (lines 312-315).

My specific comments follow, but I think this paper has a lot of potential to drive how we think through sampling and forest inventory methodology. I applaud the creativity of the researchers.

Thank you very much. We appreciate.

Numbers indicates line nos.

14-16: 25 ha is a lot of forest to inventory. I am already thinking of the sheer amount of folks I have to hire. We agree.

26: Define vegetation specifically as aboveground biomass Done.

28: Qualify aboveground Done.

45: These units seem wrong. Also, this is an older citation and only one citation given for what you indicate is a widely varying range. We deleted this example and cited a more recent example on uncertainty using field plots (lines 48-49).

30-55: In general good content, but the case needs to be made why uncertainties in rain forests are potentially higher than other forests. That would add to this section. We added a section in which we compare sampling strategies of tropical forests with those from temperate forest as (see Line 39-44).

Figure 1 : Maybe flip the scale. Done.

Figure 3: What do you mean by accurate here? The caption of figures 3-6 were revised to clarify the term.

* A point. . ..many inventory plots on the ground are circular, but I don't see specifically (unless I have missed it) but are you using circular or square estimation here? There are some deep literature that may be consulted here about the differences Lindsey, A. A., Barton Jr, J. D., & Miles, S. R. (1958). Field efficiencies of forest sampling methods. Ecology, 428-444.

We are using square plots as we are limited by the spatial resolution of the biomass map. We added this point to the methods (lines 97-99).

228-232: These section could be revisited to ensure clarity in how the results are framed. Thank you for this comment. We have revised this section (lines 262-264) and added information on the study of Marvin et al. (2014).

234 – What do you mean by sampling effort increase w/ smaller sample size? We paraphrased this sentence to clarify the statement. (lines 265-267).

Literature

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An analysis of forest biomass sampling strategies across scales

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Abstract. Tropical forests play an important role in the global carbon cycle, as they store a large amount of carbon in their biomass. To estimate the mean biomass of a forested landscape, sample plots are often used, assuming that the biomass of these plots represents the biomass of the surrounding forest.

In this study, we investigated the conditions under which a limited number of sample plots conforms to this assumption. Therefore, minimum number of sample sizes for predicting the mean biomass of tropical forest landscapes were determined by combining statistical methods with simulations of sampling strategies. We examined forest biomass maps of Barro Colorado Island (50 ha), Panama (50,000 km^2), and South America, Africa and Southeast Asia (~~7 million – 15 million km^2).~~ 3 – 11 million km^2). The results showed that around ~~100-200 plots~~100 plots (1-25 ha each) are necessary for continental~~- (1-~~wide biomass estimations if the sampled plots are ~~spatially~~ randomly distributed.

~~The~~ . However, locations of ~~the~~ current inventory plots ~~in the tropics and the data obtained from remote sensing~~ often do not meet this requirement~~. Considering the typical aggregation of~~ , e.g. as their sampling design is based on spatial transects among climatic gradients. We show that these ~~plots considerably increase the minimum sample size~~non-random locations lead to a much higher sampling intensity required~~. In the case of~~ (up to 54,000 plots for accurate biomass estimates for South America~~, it~~). Though the sample plots needed can ~~increase to 70,000 plots.~~be reduced using large distances (5 km) between the plots within transects.

We applied also novel point pattern reconstruction methods to account for aggregation of inventory plots in known forest plot networks. Results implied that current plots networks can have clustered structures that reduce the accuracy of large-scale estimates of forest biomass. To establish more reliable biomass predictions across South American tropical forests, we recommend more spatially randomly distributed inventory plots~~. If samples are generated by remote sensing, distances of more than 5 km between the measurements increase the reliability of the overall estimate, as they cover a larger area with minimum effort. The use of a combination of remote sensing data and field inventory measurements seems to be a promising strategy for overcoming sampling limitations at larger scales.~~ (minimum 100 plots). The precision of forest attribute estimates depends on the sampling intensity and strategy. This should be considered.

1 Introduction

For a better understanding of the global carbon cycle, reliable estimations of ~~carbon stocks~~aboveground biomass in vegetation have become increasingly important (Broich et al., 2009; Malhi et al., 2006; Marvin et al., 2014), especially for tropical forests, as they store more carbon in biomass than any other terrestrial ecosystem (Pan et al., 2011). Current biomass mapping approaches are based on forest field inventory plots ~~(Chave et al., 2003; Lewis et al., 2004; Lopez-Gonzalez et al., 2014; Malhi et al., 2002; Mitchard et al., 2014)~~(e.g., Chave et al., 2003; Lewis et al., 2004; Malhi et al., 2006; Mitchard et al., 2014) or remote sensing measurements ~~(Asner et al., 2013; Baccini et al., 2012; Saatchi et al., 2011) and involve statistical approaches (e.g.,~~(e.g., Asner et al., 2013; Avitabile et al., 2016; Baccini et al., 2012; Saatchi et al., 2015) and involve statistical approaches (e.g., Malhi *et al.* (2006)) or vegetation modeling (e.g., Rödig *et al.* (2017)). Remote sensing-derived maps have a typical spatial resolution of 100 - 1000 m and capture the biomass of large landscapes or even entire continents ~~(Asner et al., 2013; Baccini et al., 2012; Saatchi et al., 2011).~~(Asner et al., 2013; Avitabile et al., 2016; Baccini et al., 2012; Saatchi et al., 2011). In contrast, biomass maps based on field inventories have a higher resolution so that the ~~biomass~~local distribution ~~of local regions~~ in biomass can be described in detail~~. To combine the advantages of the two methods, we may transfer insights from field plots to high-resolution large-scale biomass maps~~.

However, the ~~extrapolation of~~biomass estimation of large forest landscapes by field inventory plots (typically between 0.25 and 1 ha) ~~to larger areas~~ poses several challenges~~,~~ in the tropics~~, where~~. Firstly, field inventory campaigns of species-rich, densely grown tropical forests are costly and labor intensive, resulting in a much smaller number of available plots than in temperate and boreal regions (Schimel et al., 2015). ~~In South America, for example, some studies created biomass maps of the whole Amazonian forest area (more than 1 billion ha) based on a very small percentage of investigated forest area (400 ha~~Currently, tropical forests are sampled with less than one plot per 1000 km^2; a density that is up to 15 times less than those that can be found in temperate zone (Schimel et al., 2015). For instance, the US national forest inventory includes more than 125,000 forest plots (Smith, 2002). This corresponds to 40 plots per 1000 km^2 In contrast, investigations of the South American Amazonian forest are often based on less than 500 forest plots (0.05 plots per 1000 km^2) (Lopez-Gonzalez et al., 2014; Mitchard et al., 2014)~~. Moreover, the locations~~ including a highly debated sampling error (Marvin et al., 2014; Mitchard et al., 2014; Saatchi et al., 2015).

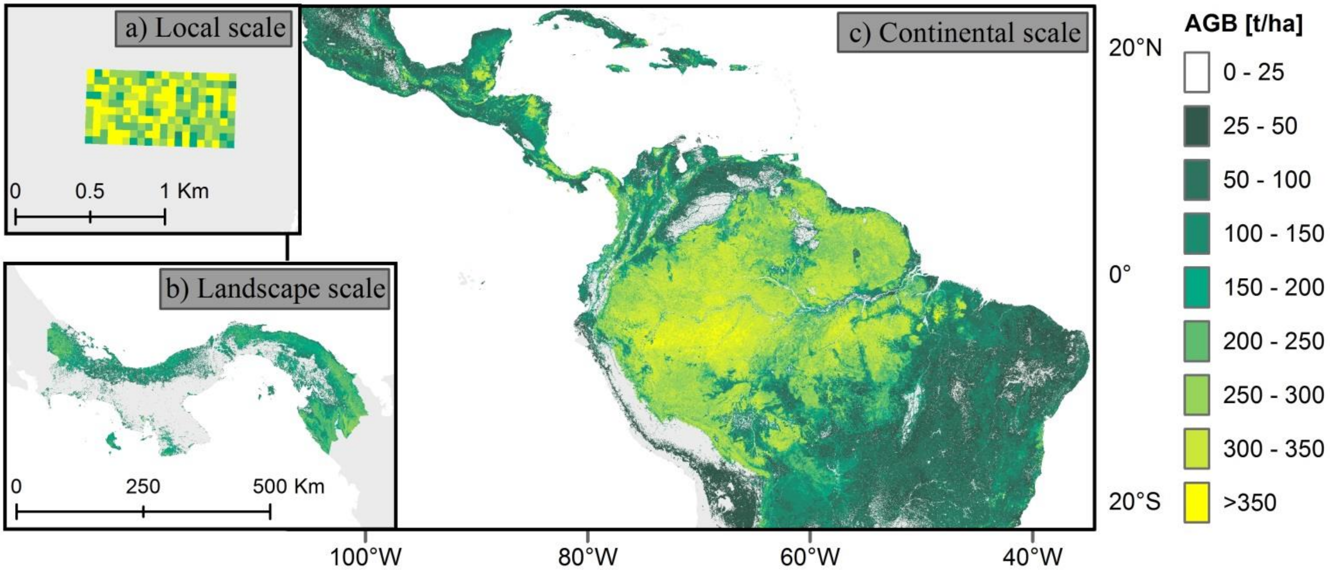
Secondly, establishments of forest plots are often ~~influenced~~limited by accessibility ~~because some regions are excluded for~~e.g., due to topographic, logistic or political reasons (Houghton et al., 2009; Mitchard et al., 2014). Even if plots are representative ~~of~~for the landscapes ~~they are located in~~ (Anderson et al., 2009), extrapolations from clustered plot networks to larger scales can be biased (Fisher et al., 2008). Consequently, ~~related estimates~~biomass estimations can include large uncertainties~~;~~, e.g.~~,~~. like estimates of the total biomass of the Amazon (93 ± 23 PgC, based on 227 forest ~~vary by~~ plots) include uncertainties of more than ~~100 %, from 38.9 to 93 PgC t/ha~~25% (~~Houghton~~Malhi et al., ~~2001~~2006).

A first step to ensure reliable extrapolations of forest biomass from field plots to large scales is to determine how many plots would be necessary to accurately estimate mean biomass on a ~~landscape~~regional scale. Previous studies suggested that for regions of about 1,000 ha, 10 - 100 sampled one-hectare plots would be necessary (Marvin et al., 2014). However, most investigations assume that plots or biomass are distributed randomly in space (Chave et al., 2004; Fisher et al., 2008; Keller et al., 2001; Marvin et al., 2014) and therefore do not consider a possible bias due to the choice of sampling strategy. The selected sampling design can significantly influence uncertainty and, consequently, the number of sample plots required (Clark and Kellner, 2012)~~. For example, in a study of different sampling designs, about 20 stratified sample plots of 0.5 ha were recommended for a region in Costa Rica (study site of 600 ha, Clark and Clark, 2000).~~. A deeper understanding of how the choice of sampling design affects the number of plots required and the influence of the size of the plots is still missing.

In this study, we present a novel simulation approach for determining the number of plots necessary across scales ~~and~~ answering the following questions: (I) How many sample plots are necessary for forest biomass estimations in South America and what is the role of the sampling strategy? (II) What is the influence of scale on the sampling design?

More specifically, we analyze different sampling strategies for biomass in tropical forests at different scales: 50 ha (Barro Colorado Island), 50,000 km^2 (Panama, (Asner et al., 2013)) and ~~15~~11 million km^2 (South America, (Baccini et al., 2012)). Following the scenario of a "virtual ecologist" (Zurell et al., 2010), we investigate through Monte Carlo simulations and analytical investigations the plot size and sample size that are necessary for accurate biomass estimations. Furthermore, we simulate nonrandom sampling strategies that imitate measurements ~~via remote sensing~~of transects and real-world forest inventories.

2 Methods



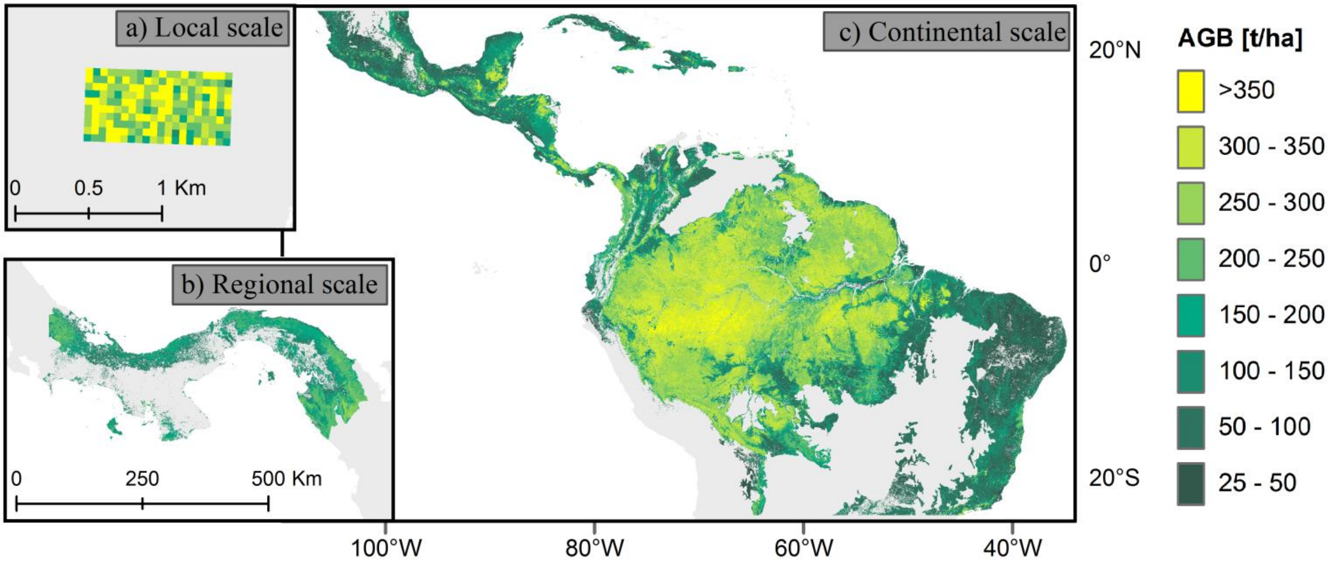


Figure 1 Forest aboveground biomass (AGB) maps used for the study. **(a)** Biomass map of a forest plot on Barro Colorado Island (50 ha, 50 m resolution~~ (Condit et al., 2012)~~). **(b)** Biomass map for Panama (~50,000 km^2, 100 m resolution~~ (Asner et al., 2013)~~). **(c)** Biomass map for South America (~11~~5~~ million km^2, 500 m resolution~~ (Baccini et al., 2012)~~). For this study, we excluded all areas covering grasslands, savannas and shrublands.

2.1 Biomass maps at different scales

We focus on three forest biomass datasets for the South American tropical region covering different scales (Fig. 1). For an analysis at the local scale, a biomass map of the Barro Colorado Island forest in Panama was applied (BCI, 50 ha) with resolutions between 10 m and 100 m. The map was based on the forest inventory of 2010 (Condit et al., 2012), which included measurements of all trees with a stem diameter greater than 1 cm (Condit, 1998). The aboveground biomass (AGB) per plot was determined using allometric relationships ~~(~~(see Supplements of Knapp, Fischer and Huth (2018)~~).~~ for details). ~~Landscape~~Regional-scale analysis was carried out using a carbon density map of Panama that was derived from Airborne Light Detection and Ranging (LiDAR) measurements from 2012, in combination with field measurements and satellite measurements (Asner et al., 2013). The AGB values for this study were calculated by multiplying the carbon values by a factor of two. We aggregated the AGB map from a 100 m resolution to resolutions of 200 m, 300 m, 400 m and 500 m. ~~For this purpose, between 4 and 25 plots from the original map were averaged.~~ If ~~these plots~~aggregated pixels covered ~~both~~a mixture of forest and non-forest areas, we assumed the non-forest areas to have a biomass of zero. ~~If the plots only covered nonforest areas, the aggregated plot was still considered as a nonforest area.~~

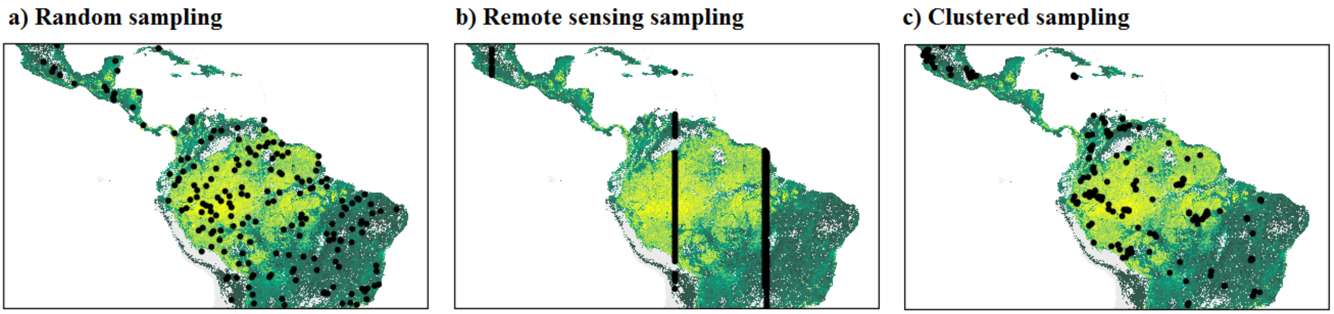
At the continental scale, we utilized a biomass map covering South America, Africa and South East Asia with a spatial resolution of 500 m ~~for South America was investigated (~15 Mio km^2,~~ (Baccini et al., 2012)~~), which was mainly derived from space-based LiDAR (observations from years between 2008 and 2010) and covered regions of the American continent between 23.4378° north latitude and 23.4378° south latitude.~~ . Biomass values of this map give information on the aboveground vegetative biomass in the time period from 2008 to 2010 and were derived using a combination of MODIS data, LiDAR measurements and field data. For our analysis, we combined this biomass map with a biome map (Dinerstein et al., 2017) and excluded all areas that covered grasslands, savannas and shrublands as well as areas with an aboveground biomass of less than 25t/ha. In order to that, remaining areas could be assigned to one of the following four tropical and subtropical forest biomes: (a) Dry broadleaf forests (b) Moist broadleaf forests (c) Coniferous forests and (d) Mangroves.

Based on ~~this map, we constructed a~~ the continental forest biomass map of South America at a 500 m resolution, we constructed an additional biomass map of South America~~–~~with a 100 m resolution using two different downscaling approaches (for details, see S3). The downscaling relationships were derived from the Panama map by upscaling this map from 100 m to 500 m resolution.

~~. For downscaling in this case, we used relationships derived from the Panama maps at 100 m and 500 m resolutions (for details, see S3). In addition, we examined AGB maps for Africa (~12 Mio km^2) and Asia (~7 Mio km^2) derived from Baccini *et al.* (2012) with a 500 m resolution (Table S1). All maps were simplified by excluding nonforest plots and plots with biomass values of less than 25 t/ha.~~

2.2 Simulated sampling strategies

We investigated three different sampling strategies: (a) random sampling (b) ~~remote sensing~~transect sampling and (c) clustered sampling (Fig. 2), with different sample sizes. For example, for analysis of the BCI forest, we divided the 50 ha biomass map into 200 square plots with a size of 50 m x 50 m~~.~~ (note that results could slightly differ if plots were circular (Alton A. Lindsey, James D. Barton, 1958)). Then, we ran simulations with sample sets containing one sample (0.25 ha), two samples (0.50 ha), and so forth until we reached a sample size of 100 samples (25 ha, half of the study area). For large-scale investigations, we analyzed sample sizes of up to 5,000 plots for Panama and 200,000 plots for South America.



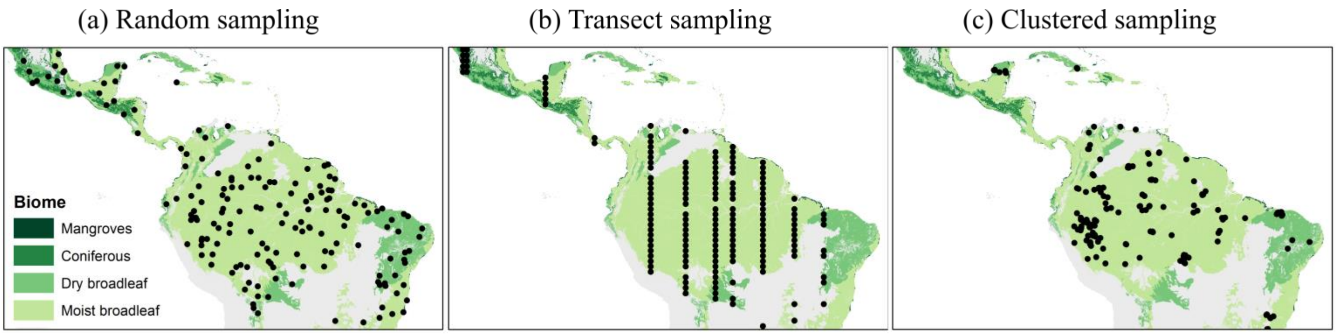


Figure 2 Examples of different simulated sampling strategies~~. Aboveground biomass samples based on the map of~~ for South America (~~AGB values are indicated by different~~ colors~~; for~~ indicate the ~~legend, please see Fig. 1).~~tropical biome)). Each black dot represents the location of one selected plot (25 ha). **(a)** Randomly distributed plots. **(b)** ~~Remote sensing~~Transect samples (shown are strips with distances of ~~50~~100 km between the plots). **(c)** Clustered samples (reconstructions of PP4).

2.2.1 Random sampling

Analysis of random sampling was performed using Monte Carlo simulations. For every map, we selected sampling plots at randomly selected positions (without replications) until the sample set reached the desired sample size. ~~Associated results were additionally determined analytically~~Random sampling is the only strategy where we can assume that the spatial autocorrelation of the map does not influence analytical analysis using the central limit theorem (Supplement S1).

2.2.2 ~~Remote sensing~~Transect sampling

~~For the remote sensing~~Transect sampling mimics sampling strategies~~, we tried to mimic typical features of airborne or satellite measurements,~~ used whenever plots should cover different gradients (e.g., ~~assuming~~climate or soil gradients). In this case, field inventory plots are established in a straight line. In our simulation approach, we assume for simplification North-South transects that ~~a satellite is flying over a direct north-south track. Starting~~start at a randomly selected position of the map~~, we selected plots with a fixed distance in a north-south direction (while nonforest plots were excluded). In this study, we focused on~~. Within one transect, the plots have regular distances ~~between 500 m and~~ of 0.5 km~~. When~~, 1 km or 5 km. Whenever the ~~plot selection~~transect reaches the southern end of the map, ~~the algorithm starts again at the northern border on~~ a new randomly selected north-south ~~track.~~ transect is chosen starting at the northern border.

The analysis of Panama was conducted by selecting plots of 1 ha (map with 100 m resolution). For South America, we selected plots of 25 ha (map with 500 m resolution). To ~~test whether the results were influenced by the direction of the remote sensing flight tracks, we also tested west-east instead of north-south tracks. With this strategy, we tried to~~ explore ~~how~~if the north-south climatic gradient influences the results~~.~~ , we also tested west-east instead of north-south tracks. However, the sampling performance remained similar (i.e., the probability of estimating the mean biomass accurately did not change significantly compared to north-~~east~~south tracks).

2.2.3 Clustered sampling

The clustered sampling approach mimics the spatial clustering of real-world field inventory networks. To this end, we reconstructed the spatial pattern of the plot networks of four studies that estimated forest biomass, including Houghton *et al.* (2001), PP1; Poorter *et al.* (2015), PP2; Malhi *et al.* (2006), PP3; and Mitchard *et al.* (2014), PP4 and analyzed them separately regarding the South American map with a resolution of 500 m (25 ha plot size). After removing duplicate locations within the 500 m grid as well as plots that are located in grasslands, savannas or shrublands (according to Dinerstein et al., (2017)) ~~,~~the number of plots per network ranged between ~~25 and 189.~~23 and 167. To generate 1,000 plot networks with similar spatial configurations as the original ones, we applied the method of pattern reconstruction (Wiegand, He and Hubbell (2013); software "Pattern-Reconstruction"). This annealing method produces stochastic reconstructions of an observed point pattern that show~~s~~ the same spatial characteristics as the observed pattern, as quantified by several point pattern summary functions (for details see S2).

2.3 Determining the minimum sample size

For each map and each sample size n, we calculated the sampling probability P_n, which quantifies how often the mean of a sample equals the mean of the underlying “true” biomass distribution (under a given accuracy) as the relative frequency out of 1,000 sample sets. For each sample set, the mean biomass ($\overline{X_{i,n}}$ in t/ha) was estimated, where i is the sample set number, and n is the sample size. $\overline{X_{i,n}}$ was then compared with the “true” mean biomass, μ [t/ha], of the underlying biomass map. A sample set was assumed to be ~~acceptable~~accurate if $\overline{X_{\iota,n}}$ was within μ ±10 %. The sampling performance can be assessed as follows:

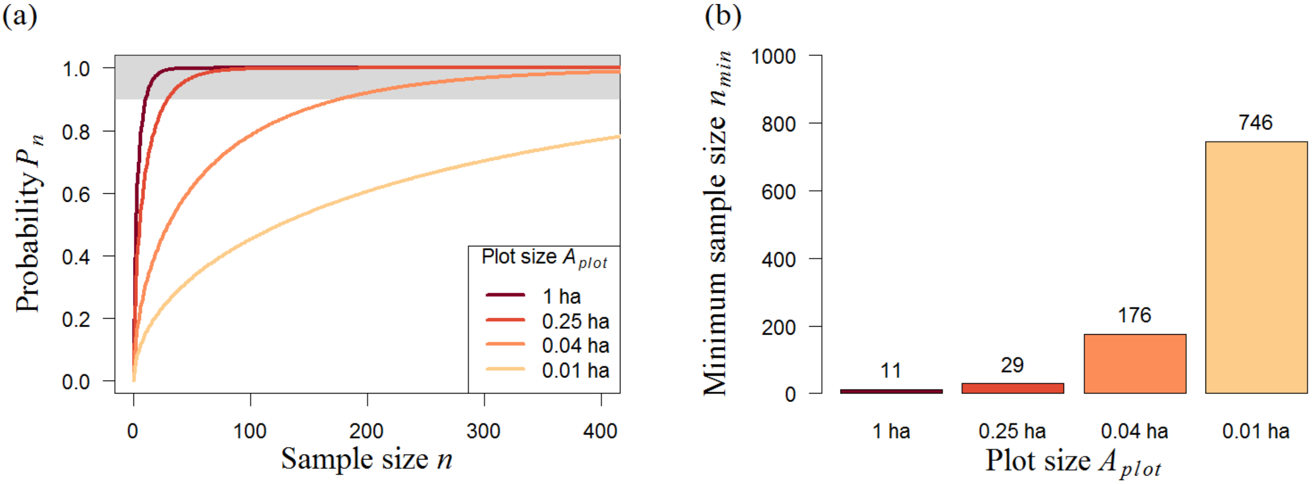
$$P_n \cong \frac{1}{1000}\sum_{i=1}^{1000} w_i\,,\ \text{with}\ w_i = \begin{cases} 1, if\ \dfrac{|\overline{X_{\iota,n}} - \mu|}{\mu} \leq 0.1 \\ 0, if\ \dfrac{|\overline{X_{\iota,n}} - \mu|}{\mu} > 0.1 \end{cases}$$

P_n typically increases with the sample size from 0 (no sample could represent the mean biomass) to 1 (all samples could represent the biomass). We defined n_{min} as the minimum sample size n, at which P_n reaches 90 %. The minimum sampling area, a_{min}, is calculated by multiplying the number of plots, n_{min}, by the plot size.

Results

3.1 Random sampling

3.1.1 Local scale (Barro Colorado Island)



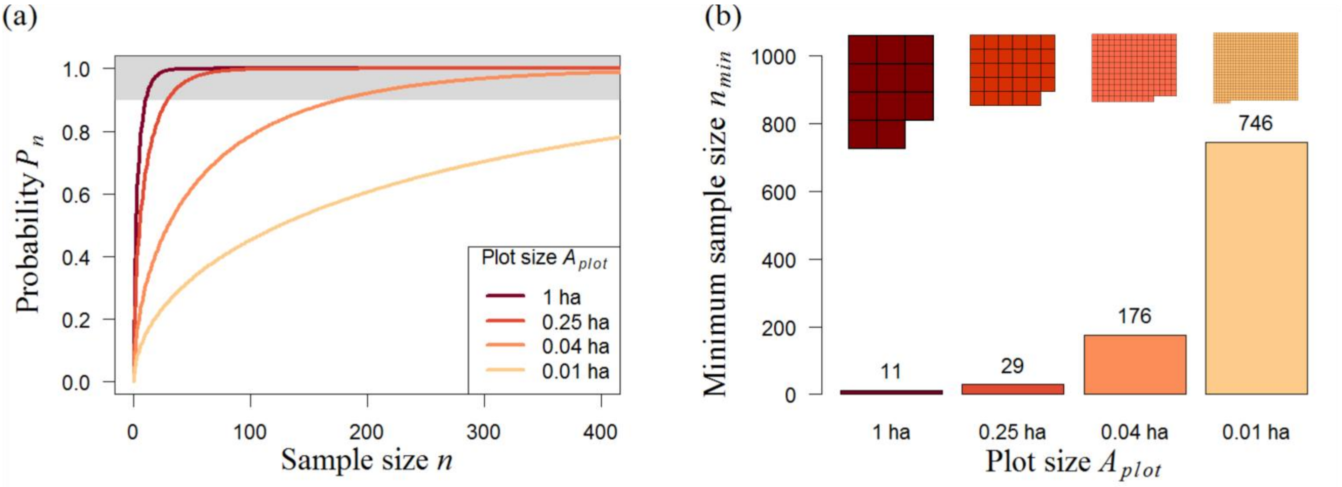


Figure 3 Analysis of different random sampling strategies for the Barro Colorado Island forest (BCI, 50 ha). **(a)** Analytical results showing the number of plots and probability $\boldsymbol{P_n}$ that the mean biomass of those plots reflects the mean biomass of the forest (for details, see Methods). We consider strategies using 0.01-1 ha plots (plot size, represented by line colors). The upper boundary (~~gray) indicates~~grey) marks sample sizes with ~~accurate~~at least 90% chance to meet the mean biomass ~~estimations ($\boldsymbol{P_n \geq 90}$ %, see Methods for details).~~of the original biomass map. **(b)** Necessary number of plots, $\boldsymbol{n_{min}}$, to estimate the biomass ~~accurately (plot sizes are displayed below~~reliably (minimum sample size from samples with $\boldsymbol{P_n \geq 90}$ %). Shapes above the bars~~).~~ represent the necessary sampling area $\boldsymbol{a_{min} = A_{plot} \cdot n_{min}}$~~.~~

The ~~results for a~~analysis of the 50 ha biomass map (BCI) ~~showed~~show the expected result that samples with larger plot sizes produce more accurate biomass estimates (Fig. 3a). ~~A~~For instance, a randomly chosen 0.01-ha plot has a probability (P_n) of 5 % of representing the mean biomass of the whole BCI forest, but if the plot has an area of 1 ha, P_n reaches 40 %. The size of the plots also affects the minimum number of plots required (n_{min}~~).~~) for reliable biomass estimates (biomass estimates that have at least a 90% chance to meet the mean biomass of the original biomass map). For small plots (plot size ≤ 0.04 ha), n_{min} decreases significantly (Fig. 3b). While only 11 one-hectare plots are needed to ~~measure~~estimate the biomass ~~correctly~~, the number of plots increases to 176 if the plot size is 0.04 ha (20 m x 20 m). However, the minimum total area of the samples (a_{min}) remains similar (Table 1, BCI); i.e., it makes no difference in sample performance whether the samples are taken from 29 plots of 0.25 ha each or 746 plots of 0.01 ha each, as an area of about 7 ha is sampled in both scenarios. Therefore, the most efficient sampling strategy for the 50 ha scale would involve 0.25 ha plots, as greater plot sizes would result in a greater total sampling area (a_{min}), and smaller plot sizes would simply increase the number of plots.

3.1.2 ~~Large~~Regional scale (Panama ~~and South America)~~)

~~In the last section, random sampling of a small homogeneous forest area was evaluated. In the next step, we analyzed the sampling results in large heterogeneous landscapes and whole continents. We analyzed~~Analyzing the biomass map of Panama (50,000 km^2) by using plot sizes between 1 ha and 25 ha (Table 1, Panama~~).~~), we found that the minimum sample size ranges between 70 and 74 plots. In contrast to the BCI analysis, plot size has no significant influence on the minimum sample size~~ in this case, since the results range between 70 and 74 plots. Larger plots lead to a slight increase in the number of plots needed~~. However, the total sampling area (a_{min}) increases from 70 ha to 1850 ha for different plot sizes. The most efficient sampling strategy at ~~the landscape~~this scale is therefore to sample 70 plots of 1 ha each.

~~For the biomass map of~~ 3.1.3 Continental scale (South America ~~(15)~~)

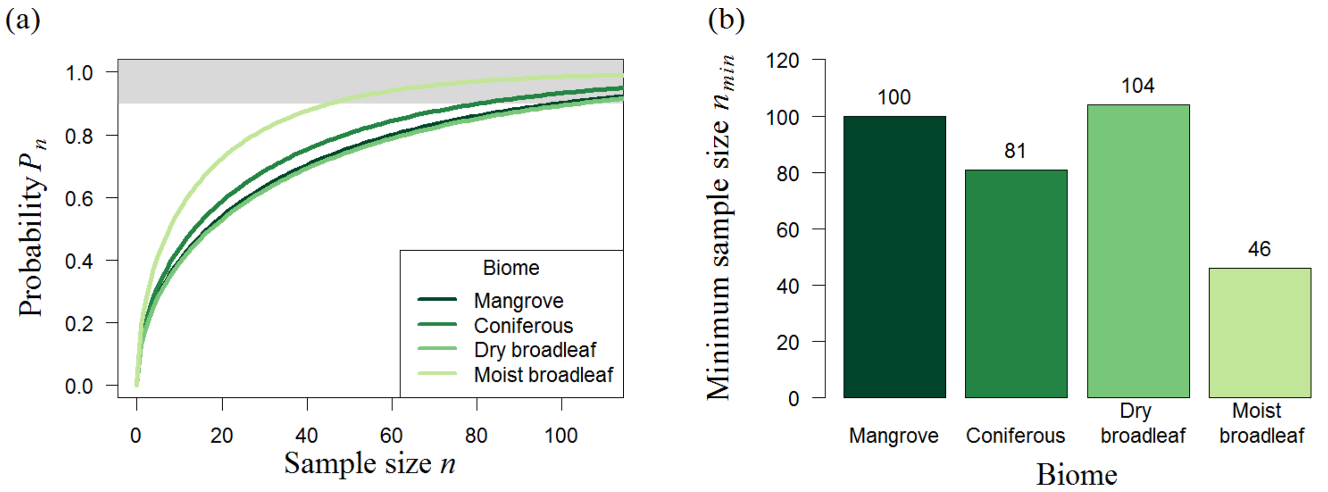


Figure 4 Results of random sampling for different biomes of South America. (a) Analytical results showing the number of plots and probability (P_n) that the mean biomass of those plots reflects the mean biomass of the forest biome (for details, see Methods) The upper boundary (grey) marks sample sizes with at least 90% chance to meet the mean biomass of the original biomass map. **(b)** Necessary number of 25 ha plots plots, n_{min}, to estimate the biomass for South America forest biomes reliably (minimum sample size from samples with $P_n \geq \mathbf{90}$ %; displayed above the bars).

We found that the needed sampling number does not depend on the total forest area of biomes when samples are chosen randomly (Fig. 4). Mangrove forests (100 plots for 90,000 km^2) are the least abundant biome in South America but require a similar number of samples as dry broadleaf forest (104 plots for 2 Mio km^2). Furthermore, the minimum sample size does not increase compared to the Panama analysis(Table 1), e.g., plot number estimations for South America moist broadleaf forest (46 plots at 500m resolution) is even 35 % less than for the Panama forest (74 plots at 500m resolution; mainly consisting on moist broadleaf forest).

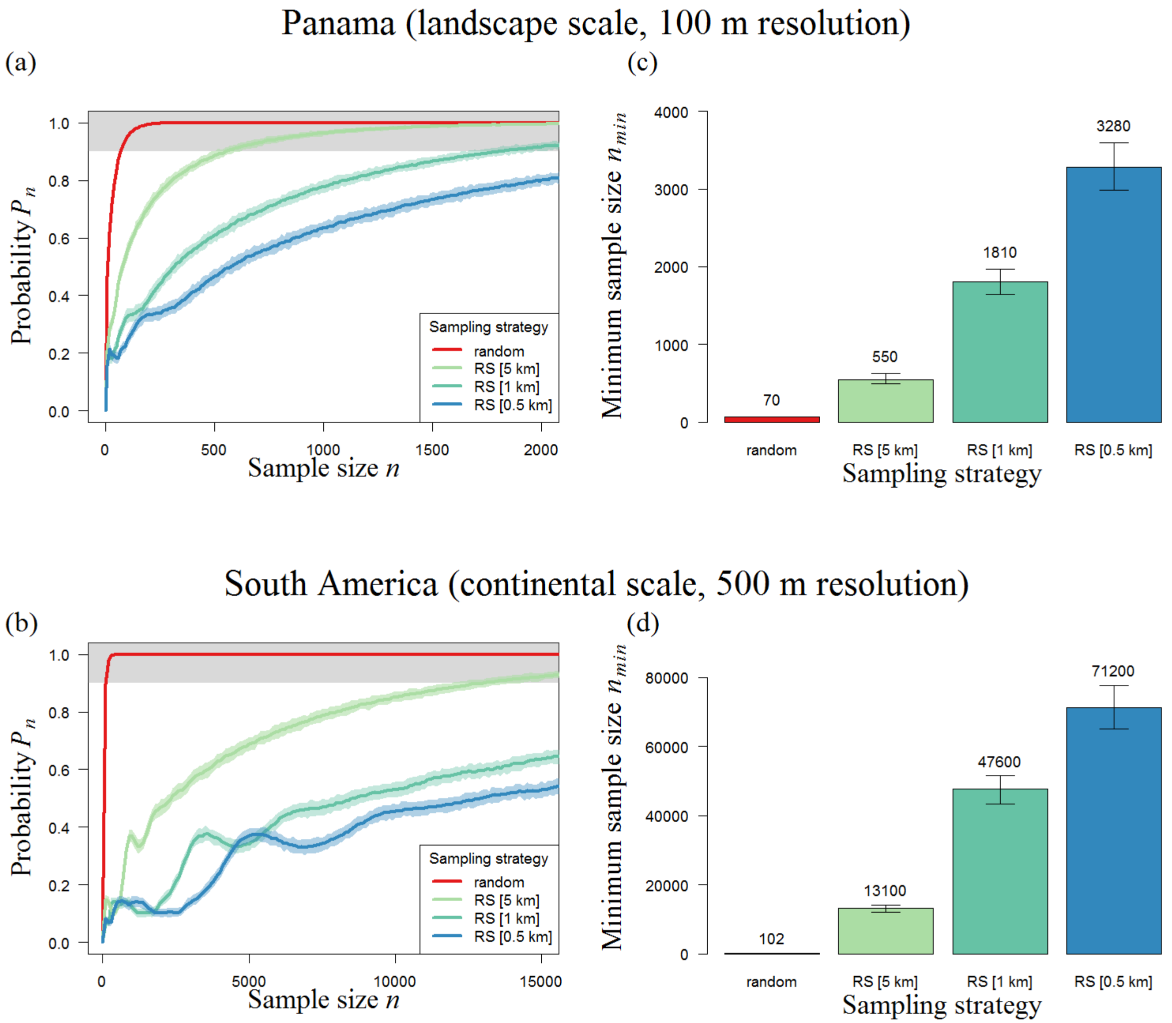
For the ~~biomass map of~~ whole South America tropical forest (11~~15~~ million km^2), ~~102~~74 plots of 25 ha are necessary to estimate the mean biomass with sufficient accuracy (Table 1, South America~~;~~ (500 m); for Africa and Southeast Asia, see Table S1). This corresponds to a total sampling area (a_{min}) of about ~~26 km^2.~~18.5 km^2. ~~For plot sizes of 1 ha each, the minimum number of plots decreases slightly to 92 plots (necessary sample area of 92 ha).~~

Using the downscaling approach D1 (see Supplements S3 for details) we found that about 70 one-ha plots would be necessary to estimate the mean biomass of the South American tropical forest (Table 1, South America (100 m)). If we assume a much higher variation of biomass values than observed in the map (downscaling approach D2, see Supplements S3), this number can rise to 121 one-ha plots.

Table 1 Analyzed forest biomass maps and the corresponding minimum sample size. The forest biomass maps for South America ~~(15,000,000 km^2, (Baccini et al., 2012)~~ (11,000,000 km^2, (Baccini et al., 2012; Dinerstein et al., 2017)), Panama (50,000 km^2,(Asner et al., 2013)) and Barro Colorado Island (50 ha, (Condit et al., 2012)) and their random sampling performance are shown. Different resolutions of the maps led to different results. The minimum sample size refers to the necessary number of plots to accurately estimate the observed mean biomass of the forest (the mean of the samples does not deviate more than 10 % from the observed mean biomass with a probability of at least 90 %). The last column shows the necessary sampling area $\boldsymbol{a_{min}} = \boldsymbol{A_{plot}} \cdot \boldsymbol{n_{min}}$.

Map (Resolution)	Plot size A_{plot} [ha]	Coefficient of variation CV [%]	Minimum sample size n_{min} [plots]	Minimum total area of samples a_{min} [ha]
South America (500 m)	25	~~61.34~~51.98	~~102~~74	~~2550~~1850
South America (100 m)	1	~~58.16~~50.63	~~92~~70	~~92~~70
Panama (500 m)	25	52.22	74	1850
Panama (400 m)	16	51.97	74	1184
Panama (300 m)	9	51.68	73	657
Panama (200 m)	4	51.27	72	288
Panama (100 m)	1	50.77	70	70
BCI (100 m)	1	19.32	11	11
BCI (50 m)	0.25	32.57	29	7.25
BCI (20 m)	0.04	80.55	176	7.04
BCI (10 m)	0.01	165.95	746	7.46

3.2 ~~Remote sensing~~Transect sampling



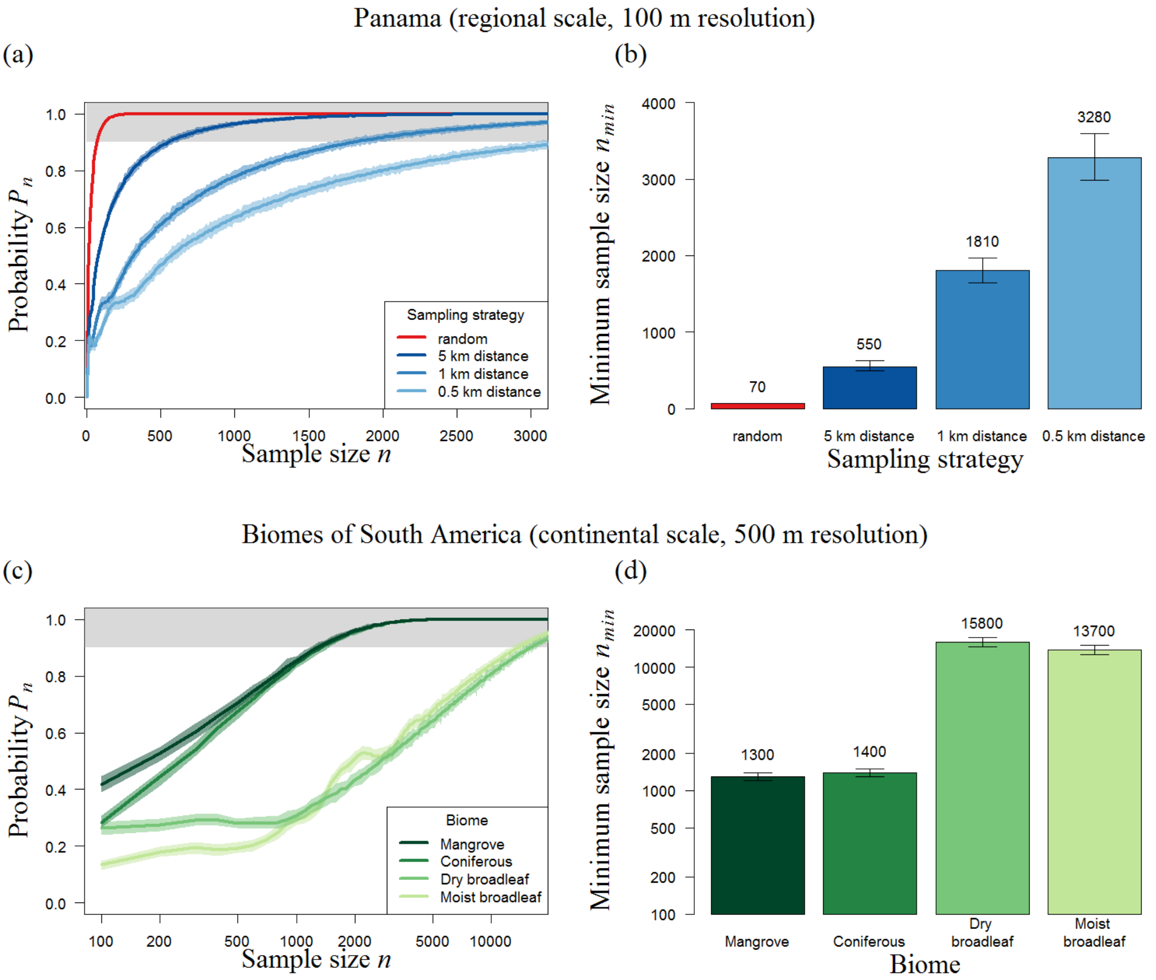


Figure ~~4~~5 Results of ~~remote sensing (RS)~~transect sampling for (a-b) Panama and (c-d) forest biomes in South America. Left: Simulation results showing the number of plots and probability (P_n) that the mean biomass of those plots reflects the mean biomass of the forest (for details, see Methods). **(a)** We focus on three~~ RS~~ strategies using distances of 500 m, 1 km and 5 km between plots (shown in blue) and compare them to random sampling (red). **(b)** Results for different biomes of the American tropical forest using a distance of 1 km. The area around each line indicates the 95 % confidence intervals of 100 repetitions (total of 1000*100 runs for each sample size). The upper boundary (~~gray) indicates~~grey) marks sample sizes with ~~accurate~~at least 90% chance to meet the mean biomass ~~estimations ($P_n \geq$ 90 %).~~of the original biomass map. Right: Necessary number (n_{min}) of 1 ha plots for Panama and of 25 ha plots for biomes of South America (error bars show the 95 % confidence intervals of 100 repetitions).

~~Sampling strategies with a specific spatial structure, such as those generated by aircraft or satellites, are tested by remote sensing (RS) sampling. The performance of nonrandom~~

The performance of non-random strategies was related to the spatial characteristics of maps (S4, Fig. S3). When the spatial clustering of the BCI forest biomass map is analyzed at the scale of 50 m, the obtained spatial biomass distribution is comparable to a random configuration; thus, the design of the sampling strategy has no influence on the results for this local forest area. For Panama and South America, the biomass is distributed in such a way that similar biomass values are more likely to be close to each other, which leads to biased estimation of the mean biomass if the samples are close to each other (e.g., ~~RS~~transects with distances of 0.5 km between the plots). This results in differences between random ~~and remote sensing~~ sampling~~:~~ and transect sampling (Fig. 5a): compared to random sampling, ~~RS~~transect samples show a lower probability (P_n) of estimating the mean biomass of the forest accurately independent of the sample size~~ (Fig. 4).~~. For Panama, random samples based on 100 one ha plots exhibit a P_n = 95 %, while ~~RS~~transect samples are less than 60 % reliable (Fig. ~~4a). For South America, RS samples based on 100 plots (25 ha plot size) show a P_n of less than 20 % (Fig. 4b~~5a).

The results show that if the distances between the plots increase from 0.5 km to 5 km, about 80 % fewer plots are necessary for accurate estimations. Larger distances between ~~the remote sensing~~ measurements within one transect make the strategy “more random”, and it therefore performs better. ~~In summary, even with large distances between plots, RS sampling leads to higher sampling efforts than random sampling. For~~Using distances of 5 km Panama~~, an~~ requires a total sampling area of 550 ha (instead of 70 ha with random sampling) ~~is necessary~~ to estimate the biomass of the 50,000 km^2 forest with a sufficient precision (Fig. ~~4c). For South America (15 million km^2), the minimum~~5b). In summary, even with large distances between plots, transect sampling ~~size increases by a factor of 130 compared~~leads to higher sampling efforts than random sampling ~~(Fig. 4d), leading to a total sample area of about 3275 km^2.~~.

For South America (11 million km^2), transect sampling based on 100 plots (25 ha plot size) show a probability P_n of less than 40 % (Fig. S4). Using distances of 5 km, the minimum sampling size increases by a factor of 140 compared to random sampling (Fig. S4), leading to a total sample area of about 2,500 km^2.

A stratification into forest biomes does not lead to a significant reduction of the overall number of needed sample plots, since the sum of the plots needed for all single biomes (in total 32,200; Fig. 5d) is similar to plots needed for an overall forest sampling (36,000 plots; Fig. S5). However in contrast to the random sampling, the area size of each biome affects the sampling effort. Here, the large broadleaf biomes need about ten times more transect samples than coniferous or mangrove forests.

3.3 Clustered sampling

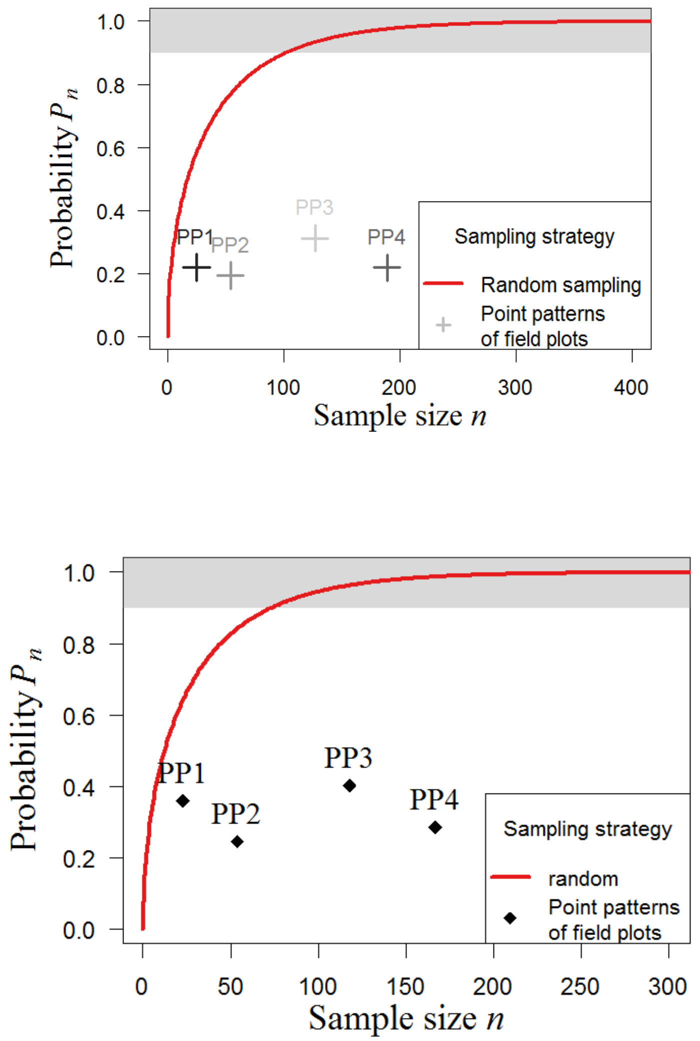


Figure ~~5~~6 Clustered sampling of biomass in South America. We tested different clustered sampling strategies using reconstructed point patterns based on the locations of existing field plots in South America (PP1-PP4). The simulation was performed with the South America map with a resolution of 500 m (25 ha plot size). Results show the probability (P_n) of accurate sampling for the spatial clustering of each point pattern (~~gray~~black crosses~~).~~, accurate means that the mean biomass of the sample does not derive more than 10% from the mean biomass of the original map). The upper boundary (~~gray) indicates~~grey) marks sample sizes with ~~accurate biomass estimations ($P_n \geq$ 90 %, see Methods for details).~~at least 90% chance to estimate biomass accurately. As a reference, the results for random sampling are shown (red line).

Samples based on forest inventory plots are often influenced by accessibility, which leads to nonrandom locations of the sample plots that are simulated under the clustered sampling approach. Here, we examine the biomass map of South America with reconstructed point patterns PP1-PP4 based on the locations of existing inventories in South America (~~25-189~~23-167 plots, see Methods). The results show that the probability (P_n) of estimating forest biomass accurately is considerably lower compared to the probability associated with random samples (Fig. ~~5~~6). All samples present less than a ~~35~~45 % chance of reflecting the real mean biomass for South America. For clustered sampling, a greater number of samples per se does not lead to better biomass estimations. The positions of the plots therefore play a crucial role. Although PP4 combines many plots of PP1 and PP3, the stochastic sampling scheme based on the spatial aggregation of plots cannot capture the biomass distribution significantly better than those based on the single datasets alone. In summary, the simulation results demonstrate that non-random strategies such as ~~remote sensing~~transect sampling and clustered sampling differ significantly from random sampling, leading to increased sampling efforts and noticeably greater sampling uncertainties.

4 Discussion

Due to the large area of tropical forest, only a few parts of the forest can be investigated in detail. Therefore, effective sampling strategies for these forests are relevant (Broich et al., 2009; Chave et al., 2004; Malhi et al., 2006; Marvin et al., 2014). The question of how many forest plots are necessary to predict forest biomass has not yet been fully answered. Thus far, sampling quality has often been determined on the basis of the assumption that samples are spatially randomly distributed (Chave et al., 2004; Fisher et al., 2008; Keller et al., 2001; Marvin et al., 2014). However, sampling at large scales in the tropics often does not fulfill this condition because in many cases, random locations are difficult to access (Wang et al., 2012). In this study, we compared different sampling strategies for tropical forests across various scales and plot sizes examining the probability to obtain the correct biomass estimate and the associated minimum sample size. Therefore, we analyzed random samples and compared them to simulated samples that are spatially clustered. Please note that in this study, we did not consider additional error sources, e.g., due to tree size measurements or allometric models, even though they are also known to influence biomass estimates (Chave et al., 2004).

~~Therefore, we simulated random samples and compared them to samples that are specified by remote sensing flight routes or spatially clustered.~~

4.1 Random sampling

Focusing on forests in South America ~~W~~we showed that independent from forest area, less than 100 ~~11-92~~ randomly distributed one-ha plots ~~(1 ha each, depending on the forest extent)~~ are necessary to estimate the mean biomass ~~for tropical forests in the South American region.~~ with sufficient precision. This result is in line with a study by Marvin *et al.* (2014) predicting minimum sample sizes between 10 and 100 plots for forest regions in Peru (1 -10 km^2). ~~in which the minimum sample sizes for biomass maps of regions in Peru (1 –10 km^2) were investigated. The results demonstrated that the main factor influencing the minimum sample size is the variability in biomass values in the map, so homogeneous Amazonian landscapes require fewer plots than heterogeneous regions in the Andes (Marvin et al., 2014).~~

By testing plot sizes between 0.01 ha and 1 ha, we demonstrated that ~~sample~~inventory plots should not be smaller than 0.25 ha because ~~sampling effort increases with smaller plot sizes~~ smaller plots tend to be considerable more heterogeneous (reflected by a significant increase of the CV) and lead to a considerable greater number of necessary sample plots. Although the coefficient of variation (CV) of the biomass distribution increases with decreasing plot size for local forests (Réjou-Méchain et al., 2014; Wagner et al., 2010), there seem to be only small effects for larger landscapes. For Panama, we even found that biomass distribution of the aggregated maps were more heterogeneous due to averaging forest with non-forest areas. To estimate the minimum sample size of a particular forest region, it might be useful to explore variability, for example, by using forest models (Zurell et al., 2010) or topography (Réjou-Méchain et al., 2014).

~~To estimate the minimum sample size of a particular forest region, it might be useful to assess variability, for example, by using forest models (Zurell et al., 2010), forest type maps (Marvin et al., 2014) or topography (Réjou-Méchain et al., 2014).~~

~~For large areas (tropical forests in South America, Africa, Southeast Asia), we obtained minimum sample sizes of about 100-200 plots (randomly distributed, 25 ha each) on each continent. We also tested larger plot sizes with a biomass map from Saatchi *et al.* (2011), but the results were similar (100-230 plots, 100 ha each). Furthermore, our tests included smaller plot sizes obtained by downscaling the South American biomass map using relationships derived from the Panama forest biomass map (50,000 km^2 forest area). The results indicated that 100 plots of 1 ha that are randomly distributed in space are sufficient for biomass estimations in South America at large scales (Table 1). Please note that we tested a simple downscaling procedure, so caution must be applied to these initial findings.~~

~~Although the coefficient of variation (CV) of the biomass distribution decreases with increasing plot size for local forests (Réjou-Méchain et al., 2014; Wagner et al., 2010), there seem to be only small effects for larger landscapes. For Panama, we even found a slight increase in CV when the plot size was changed from 1 ha to 25 ha, since the mean biomass value for the region was reduced due to averaging forest with non-forest plots.~~

For large areas (tropical forests in South America, Africa, Southeast Asia), we obtained minimum sample sizes of 74-103 plots (randomly distributed, 25 ha each) on each continent. We also tested larger plot sizes with a biomass map from Saatchi *et al.* (2011), but the results were similar (75-136 plots, 100 ha each). Furthermore, our tests included smaller plot sizes obtained by downscaling the South American biomass map using relationships derived from the Panama forest biomass map (50,000 km^2 forest area). The results indicated that 70 plots of 1 ha that are randomly distributed in space are sufficient for biomass estimations in South America at large scales (Table 1). We are aware that the biomass maps used have limitations (i.e., saturation effects from remote sensing measurements, or missing fine scale data). To investigate the sensitivity of this effect on the sampling results, we constructed a 100 m resoluted biomass map with much higher variation in biomass values than observed in the current biomass map. In this case, the number of one-ha plots that are necessary for continental estimates of the South American tropical forest increased to 121 one-ha plots (instead of 70 plots). Please note that we tested a simple downscaling procedure, so caution must be applied to these initial findings.

4.2 Nonrandom sampling

Our analysis showed that sampling efforts change significantly if samples are not random in space. For South America, non-random samples of forests are less reliable and require significantly more plots to achieve accurate biomass estimations. This means that the necessary number of plots for non-random sampling strategies (as can be found in real-world inventories ~~and RS measurements~~) cannot be assessed by Monte Carlo simulations that implicitly assume that samples are random (as in related studies of (Chave et al., 2004; Fisher et al., 2008; Keller et al., 2001; Marvin et al., 2014). Instead simulation procedures need to incorporate more advanced methods that include aggregated plot placement.

We demonstrated that a spatial autocorrelation has an effect on the sampling strategy (Legendre et al., 1989; Réjou-Méchain et al., 2014) if plots close to each other are more similar than plots located farther apart (positive autocorrelation). ~~Larger~~Result suggest that for larger regions ~~were shown~~biomass tend to be more spatially clustered ~~in terms of biomass~~ (e.g., large forest biomass ~~stocks~~ occur more frequently within the Amazon basin than in the surrounding landscape) because biomass varies due to environmental gradients and geographical reasons (Houghton et al., 2009). Therefore, the uncertainty of large-scale ~~analyses are~~estimations might be more affected by the sampling design than ~~regional estimations.~~estimates for local scales. However, also small forested regions can be spatially correlated in terms of biomass (e.g., due to management or topography) so biases can’t be excluded whenever the sampling design is not random.

~~The methods presented above can also be applied to other forest attributes (e.g., species richness and carbon fluxes) and can be adapted to other ecosystems, such as grasslands or farmlands.~~Existing plot networks may provide better estimates than suggested by a “blind” sampling using additional information (e.g. climate and soil covariates). Those covariates can be utilized e.g., to define weighting factors that enhance biomass mean estimation. To analyze this issue, the pattern reconstruction approach used in this study could include additional criteria (ideally also those used for selection of plots). If the covariates can be mapped in the entire study area, the pattern reconstruction approach can take into account the additional constraints and reject plot configurations that do not agree with these criteria.

~~Please note that in this study, we did not consider additional error sources, e.g., due to tree size measurements or allometric models, even though they are also known to influence estimates (Chave et al., 2004).~~

5 Conclusions

In summary, our study shows that the accuracy of the biomass estimates derived from samples depends considerably on the sampling strategy. Inventories are highly relevant for studying forest structure and dynamics. For South America, we have shown that more spatially randomly distributed plots are beneficial for continental-wide biomass estimations. ~~Plot~~For a given sampled area, plot size should not fall below 0.25 ha, as the variability of biomass values will strongly increase (Chave et al., 2003; Clark et al., 2001; Keller et al., 2001; Réjou-Méchain et al., 2014), and tree-level measurement errors can dominate (Chave et al., 2004). ~~Since~~

To establish forest plots randomly across South-America is challenging. On the one hand, mature tropical forests have high tree densities (Crowther et al., 2015) , so measurements are more labor intensive. On the other hand, random plot locations may lead to large distances between the plots (Wang et al., 2012) making them more difficult to access and also results in higher efforts and costs.

Some studies combine field inventories with remote sensing data to estimate the biomass of large regions (Asner et al., 2013; Baccini et al., 2012; Rödig et al., 2017; Saatchi et al., 2011) as remote sensing can sample forest regions in a short time (Houghton et al., 2009; Schimel et al., 2015)~~, a combination of remote sensing data and field inventory data seems to be a promising approach to overcome current limitations~~. The here shown transect sampling could give also hints for remote sensing based on airplane campaigns flying in straight lines over forest transects (e.g., comparable to Asner et al., 2013). The methods presented can be applied to any spatially clustered sampling technique. The sampling design is very relevant not only for forest biomass estimations, but also in view of other forest attributes (e.g., production). This should be considered when establishing forest plot networks.

Code and Data availability

Biomass data (BCI, Panama, tropics) is available in the corresponding reference. The R code for sampling simulations is available upon request from the corresponding author.

Author contribution

JH, RF, and AH conceptualized the research; JH prepared the data and ran analyses. TW supported point pattern analysis. HJD contributed to analytical solutions. JH, RF and AH prepared the first draft of the manuscript and all the co-authors contributed substantially to subsequent versions, including the final draft.

Competing interests

The authors declare no competing interests.

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Supplement of "An analysis of forest biomass sampling strategies across scales"

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S1 Theory of random sampling

Random sampling can be covered analytically by the central limit theorem, which states that whenever independent random variables are added, their sum converges toward a normal distribution irrespective of the original distribution. Suppose that $\mathrm{X}_1, \mathrm{X}_2, \dots \mathrm{X}_\mathrm{n}$ is a sequence of independent identically distributed random variables with a mean $\overline{\mathrm{X}_\mathrm{n}}$, finite expected value $\mathrm{E}(\mathrm{X}_\mathrm{i}) = \mu$, and variance $\mathrm{Var}(\mathrm{X}_\mathrm{i}) = \sigma^2$. For $n \to \infty$, the distribution function, Z_n, converges to the standardized normal distribution.

$$\mathrm{Z}_\mathrm{n} = \frac{\sqrt{\mathrm{n}}(\overline{\mathrm{X}_\mathrm{n}} - \mu)}{\sigma}$$

The probability, P_n, that $\overline{X_n} \in [0.9 \cdot \mu, 1.1 \cdot \mu]$ can be calculated as the "sample reliability", where Φ is the probability of the normal distribution, can be calculated as follows:

$$P_n = P\left(|z_n| < 0.1\sqrt{n}\frac{\mu}{\sigma}\right) = \Phi\left(0.1\sqrt{n}\frac{\mu}{\sigma}\right) - \Phi\left(-0.1\sqrt{n}\frac{\mu}{\sigma}\right)$$
$$= 2 * \Phi\left(0.1\sqrt{n}\frac{\mu}{\sigma}\right) - 1 \qquad (1)$$

The "minimum sample size", n_{min}, defines the number of samples needed under the condition that the mean of the samples does not deviate more than 10 % from the real mean biomass with a probability of at least 90 %. We determined n_{min} using Eq. (1) and the quantile of the standardized normal function, q:

$$2 \cdot \Phi\left(0.1\sqrt{n}\frac{\mu}{\sigma}\right) - 1 \geq 0.9$$

thus $$\Phi\left(0.1\sqrt{n}\frac{\mu}{\sigma}\right) \geq \frac{1.9}{2}$$

Therefore $$0.1\sqrt{n}\frac{\mu}{\sigma} \geq q(\frac{1.9}{2})$$

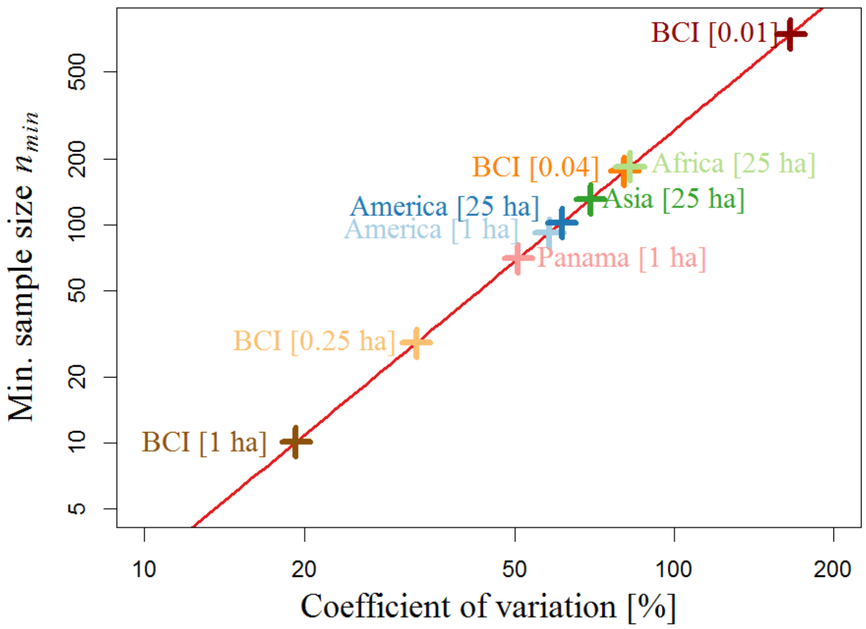
Finally, $$n \geq \frac{(q(0.95)\cdot\sigma)^2}{(0.1\,\mu)^2}$$

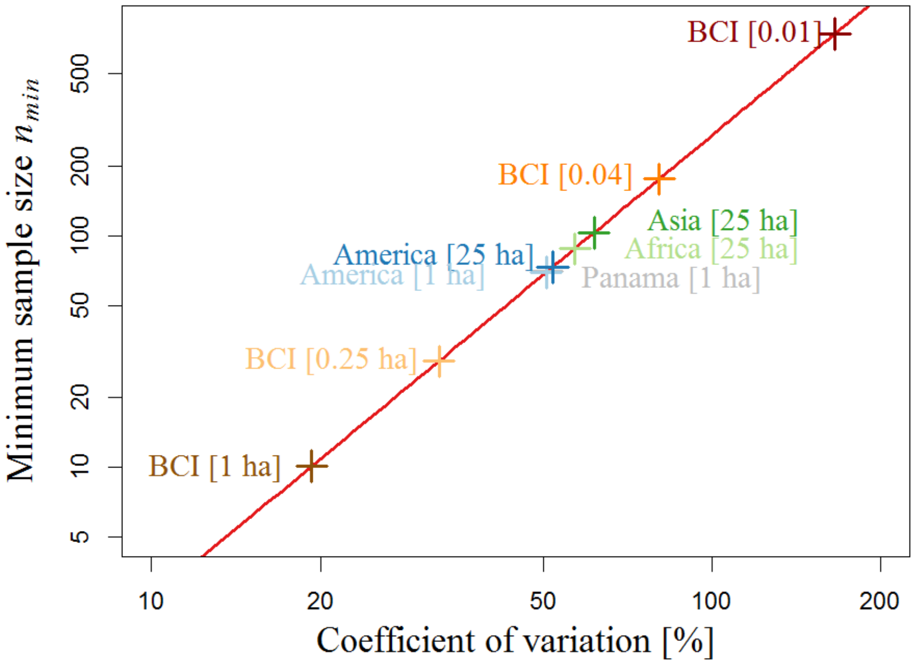
The minimum sample size here is assumed to be the minimum number of samples for which this equation is still valid.

$$n_{min} := \left\lceil \frac{(q(0.95) \cdot \sigma)^2}{(0.1\,\mu)^2} \right\rceil$$

n_{min} depends only on the mean biomass, μ, and its standard derivation, σ. In this study, this term is simplified using the coefficient of variation (CV=$\frac{\sigma}{\mu}$) as follows:

$$n_{min} = \left\lceil \frac{q(0.95)^2}{0.01} \cdot CV^2 \right\rceil \qquad (3).$$


BCI [0.01]
BCI [0.04]
Africa [25 ha]
Asia [25 ha]
America [25 ha]
America [1 ha]
Panama [1 ha]
BCI [0.25 ha]
BCI [1 ha]
Min. sample size n_{min}
5
10
20
50
100
200
500
10
20
50
100
200
Coefficient of variation [%]


BCI [0.01]
BCI [0.04]
Asia [25 ha]
Africa [25 ha]
America [25 ha]
America [1 ha]
Panama [1 ha]
BCI [0.25 ha]
BCI [1 ha]
Minimum sample size n_{min}
5
10
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50
100
200
500
10
20
50
100
200
Coefficient of variation [%]

Figure S1 Relation between the minimum sample size and the coefficient of variation (CV) of the biomass distribution. The results for random sampling of Barro Colorado Island (BCI, 50 ha), Panama (~50000 km^2~~ (Asner et al., 2013)), America, Africa and Asia (~7-15 Mio km^2 (Baccini et al., 2012)) are shown.~~), America, Africa and Asia (~3-11 Mio km^2) are shown. The analyzed biomass maps are displayed with colored crosses (with the name and plot size shown in brackets). The analytical relationship between CV and the minimum sample size has been derived from equation 3 (red line). Please note that both axes are logarithmized.

Table S1 Analyzed forest biomass maps for the tropics and their minimum sample size. Shown are the forest biomass maps for (a) South America, Africa and Southeast Asia (Baccini et al., 2012) and for (b) South America, Africa and Asia & Australia (Saatchi et al., 2011) and their random sampling performance. The minimum sample size refers to the minimum number of plots to accurately estimate the mean biomass of the forest (the mean of the samples does not deviate more than 10 % from the real mean biomass with a probability of at least 90 %). The last column shows the necessary sampling area $\boldsymbol{a_{min}} = \boldsymbol{A_{plot}} \cdot \boldsymbol{n_{min}}$.

Map (Resolution)	Map size [Mio. km^2]	Plot size A_{plot}[ha]	CV	Minimum sample size $\boldsymbol{n_{min}}$ [plots]	Minimum total area of samples a_{min} [ha]
a)					
South America (500 m)	~~15~~ 11.3	25	~~61.34~~51.98	~~102~~74	~~2550~~1850
Africa (500 m)	~~12~~3.4	25	~~82.54~~56.94	~~185~~88	~~4625~~2200
Southeast Asia (500 m)	~~7~~6.1	25	~~69.43~~61.61	~~131~~103	~~3275~~2575
b)					
South America (1000 m)	~~61~~11.4	100	~~65.83~~52.51	~~118~~75	~~118000~~75000
Africa (1000 m)	~~46~~3.8	100	~~93.44~~62.23	~~237~~105	~~237000~~105000
Asia & Australia (1000 m)	~~73~~10.7	100	~~82.63~~70.65	~~185~~136	~~185000~~136000

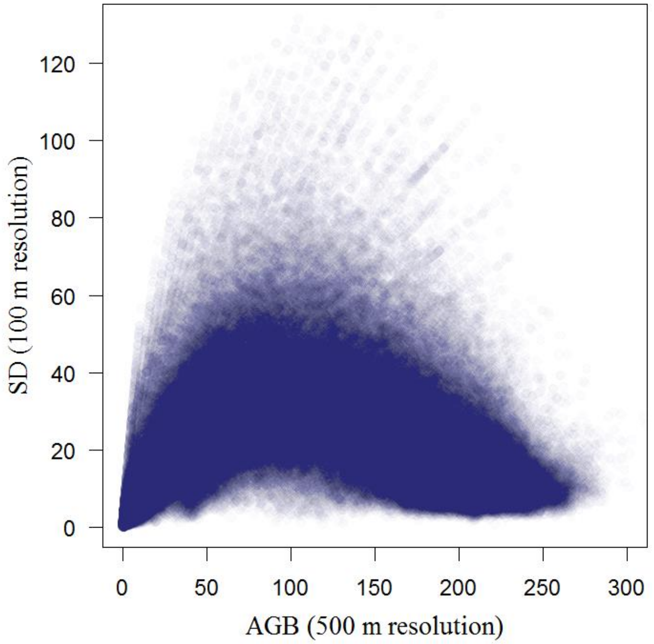
S2 Point pattern summary functions for the clustered sampling approach

For the reconstructions of the clustered locations of the inventory plots, we use several point pattern summary functions that quantify the spatial structure of the pattern within distances of up to 100 km: (I) the probabilities $p(k, r)$ that the typical point of the pattern has k neighbors within distance interval r – bin/2, r + bin/2, where bin = 500 m is the resolution of the map and r = 0.5, 2.5, 7.5, 17.5, 25, and 50 km. (II) the distribution function $D(r)$ of the distances to the nearest neighbor; (III), the average number of points at distance r from the points of the pattern given by $\lambda(2\pi r)g(r)$ where λ is the density of the pattern and $g(r)$ the pair correlation function; (IV) the average number of points at a distance r from the points of the pattern given by $\lambda K(r)$, where $K(r)$ is Ripley's K; (V) $H_s(r)$ the spherical contact distribution; (VI) the distribution functions $D_k(r)$ of the k'th nearest neighbor. For further details see Wiegand, He and Hubbell, (2013).

S3 Downscaling of the South America map

To downscale the 500 m resolution map of South America to a 100 m resolution, we used statistical relationships derived from the Panama map at 100 m and the 500 m resolution. Therefore, 25 plots from the original map (100 m resolution) were aggregated to a mean value. The standard deviation of those 25 plots at a 100 m resolution sd_{100} can then be plotted against the mean value of the plots, which can be interpreted as the aggregated value at a 500 m scale AGB_{500} (see Fig. S2 a). For the first downscaling strategy (D1) we transferred the derived relationships to the South America map. After creating classes over the AGB of 1 t/ha, each AGB value of 25 ha plot of the South American map was assigned to 25 plots of 1 ha drawing random values from a normal distribution $N(AGB_{500,}\ (sd_{100})^2)$. If the South American plot had an AGB value higher than the maximum value of Panama, the created plots were drawn from a normal distribution with the standard deviation of the maximum class. Negative biomass plots were set to zero.

We analyzed also a second downscaling strategy (D2). Here we assume that the variation of subplots increases linear with biomass (compare Figure S1). For this down-scaling strategy the linear trend resulting from AGB values smaller than 100 t/ha is continued for larger biomass values. Each AGB value of the South American map (1 pixel = 25ha) was assigned to 25 plots of 1 ha drawing random values from a normal distribution $N(AGB_{500,}\ (m*AGB_{500}+t)^2)$ with slope m and intercept t as coefficients of the linear regression.



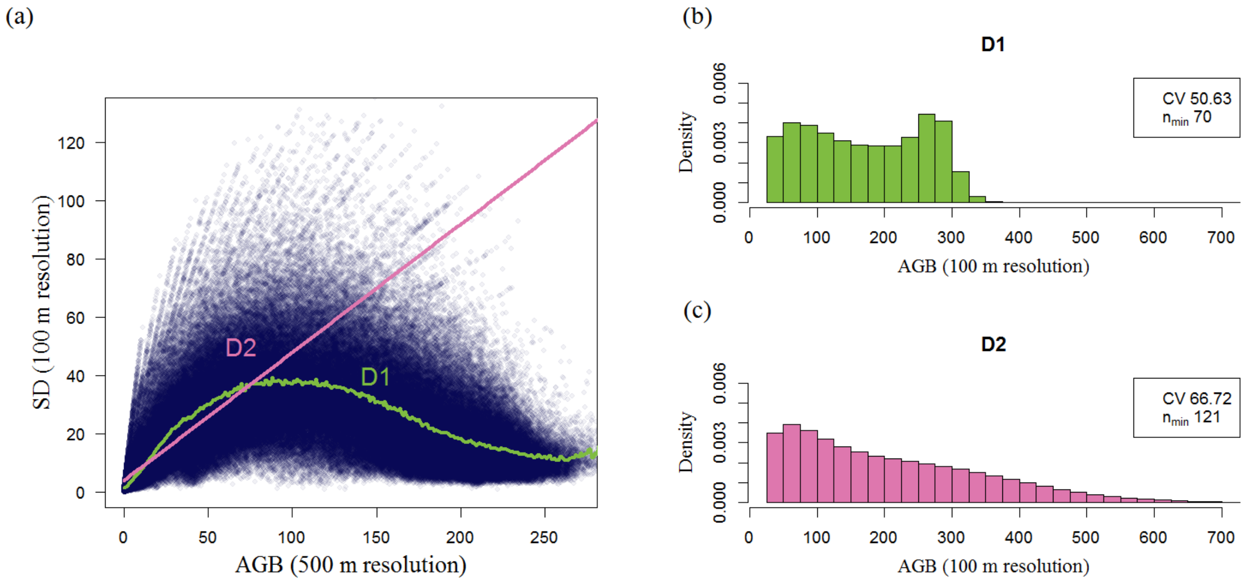


Figure S2 Comparison of downscaling approaches. **a)** Subplot heterogeneity in the Panama biomass map (500 m resolution). Shown is the aboveground biomass (AGB) of plots at a 500 m resolution (x-axis) and the standard deviation (SD) of ~~its~~the associated 25 subplots at a 100 m resolution~~.~~ (y-axis). Each dot represents one plot from the Panama map (~300,000 plots).The green line represents the downscaling approach D1, as it was implemented in the current study (Table 1). The second downscaling approach D2 shown in pink is based on an increasing linear relationship. **b-c)** Aboveground biomass distribution of South America at a 100 m resolution for the two analysed downscaling approaches. Coefficient of variation (CV) and the minimum sample size (n_{min}) of randomly chosen 1 ha plots are shown at the upper right corner for each biomass distribution.

S4 Spatial clustering of the biomass

To quantify spatial correlation structures in the biomass map we used the mark variogram $\gamma_{mm}(r)$ (Illian et al. 2008; Wiegand and Moloney 2014). Each grid cell of the map has x-y coordinates and the associated biomass value is the mark. We then consider all pairs of cells that are distance r apart and determined the mean value of the test function $t_4(m_1, m_2) = (m_1 - m_2)^2/2$ where m_1 is the biomass mark of the first cell and m_2 that of the second cell. The mark variogram has small values if the biomasses of cells that are distance r apart are in general similar to each other and large values if the biomasses are dissimilar. To test for significant spatial structure we compared the observed mark variogram to that of a null model where we randomly permutated the biomass values among the all forested cells.

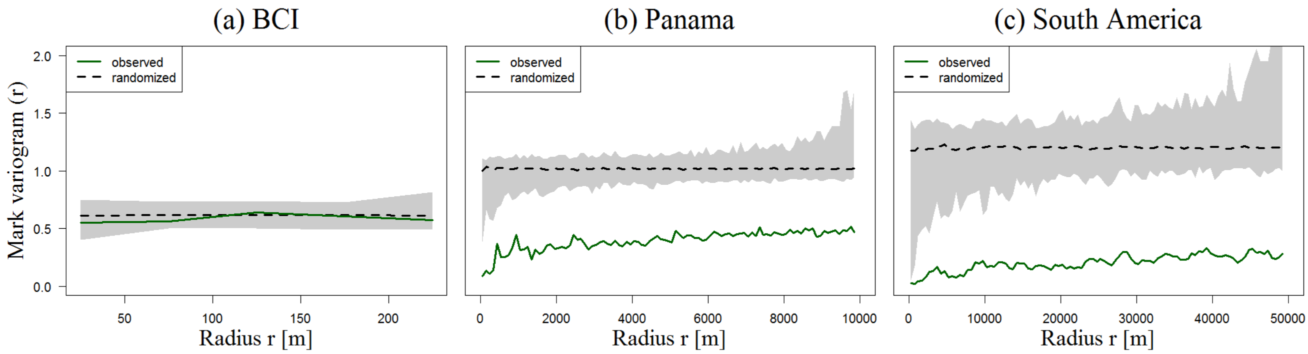


Figure S3 Spatial structure of the biomass maps. Shown is the observed mark variogram (green line, for details see Illian *et al.* (2008)) for maps of Barro Colorado Island (50 m resolution, 50 ha), Panama (100 m resolution, ~50000 km^2) and South America (500 m resolution, 15 Mio km^2). Values of the dashed black line show the expectation under the null model of random distribution of biomass (gray color displays the 99% simulation envelopes). Values below the envelopes indicate clustering (more similar biomass values at short distances than expected by the null model), values above indicate overdispersion (more dissimilar biomass values than expected by the null model, e.g., comparable to a chessboard). For further details on the method, see Wiegand and Moloney (2014). Please note that for Panama and America, we use subsets of 10,000 plots. **(a)** No significant spatial structure was observed for the 50m resolution for Barro Colorado Island (BCI)**. (b-c)** In contrast to BCI, the observed biomasses for Panama and America are below simulation envelopes, indicating biomass values are within distances of 10 km and 50 km more similar to each other than expected by a random distribution of biomasses over all forested cells.

S5 Transect sampling in South America

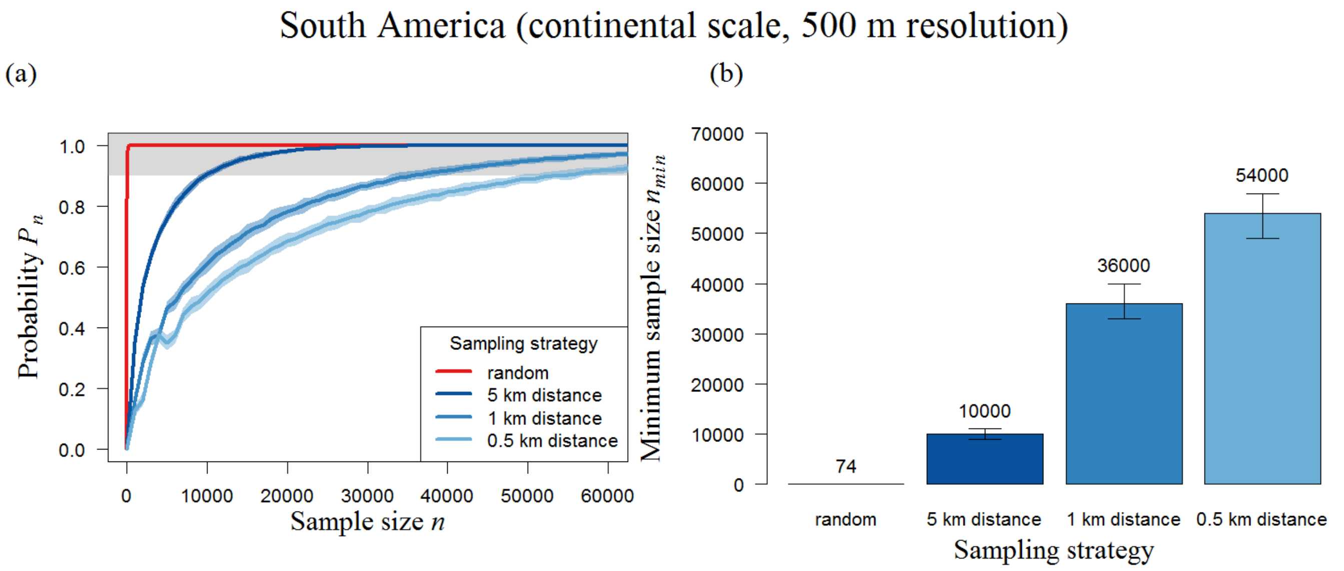


Figure S4 Results of transect sampling for different biomes of South America. **(a)** Simulation results showing the number of plots and probability (P_n) that the mean biomass of those plots reflects the mean biomass of the whole forest (for details, see Methods). We focus on three strategies using distances of 500 m, 1 km and 5 km between plots (shown in blue) and compare them to random sampling (red). The area around each line represents the 95 % confidence intervals derived from 100 repetitions (total of 1000*100 runs for each sample size). The upper boundary (gray) marks sample sizes with accurate biomass estimations ($P_n \geq 90$ %). (b) Necessary number (n_{min}) of 25 ha plots for South America (error bars show the 95 % confidence intervals of 100 repetitions).

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