**University of São Paulo
“Luiz de Queiroz” College of Agriculture**

**Estimation of volume, biomass, and carbon of coarse woody debris in
native forests in São Paulo State, Brazil**

Andréa Bittencourt Moreira

Thesis presented to obtain the degree of Doctor in Science. Program: Forest Resources. Option in: Silviculture and Forest Management

**Piracicaba
2017**

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Forest Engineer

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versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:

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I dedicate this work
to Professor Timothy Gordon Gregoire

Model of dedication and love to what he does. He goes a long way beyond what duty imposes. He is concerned with the realization of the work without leaving the human being aside. His students know they will always have a friend and a place to find support and words of wisdom. There are no questions he does not readily answer, and there is no wait for him to help find the way. His office is the temple where the wisdom lives, and there is always an open door. I count on his eternal friendship. Thank you very much for allowing my path to be part of yours.

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“A new destination
The planet of visions is calling tonight
Another thousand years seem so long
I’m just a passenger
And the ride has just begun
Moment of glory
A moment of glory called evolution”

Klaus Meine

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RESUMO

Estimativa do volume, biomassa e carbono de madeira morta em florestas nativas no Estado de São Paulo, Brasil

Este estudo buscou testar a metodologia de amostragem pela linha interceptadora (LIS), usando a inferência baseada no design, com amostragem sistemática estratificada em duas formas de transectos: linha reta e forma de cruz, ambos com três diferentes comprimentos de transectos: 100, 150 e 200 m para estimar o número de elementos, volume, biomassa e estoque de carbono de madeira morta; investigar a densidade da madeira e a concentração de carbono da madeira morta pela classe de decomposição; e, ajustar e selecionar modelos de biomassa e carbono de madeira morta, em dois tipos de floresta nativa no estado de São Paulo, Brasil: uma Floresta Estacional Semidecidual (SSF) e um Cerrado *sensu-stricto* (CSS). Em cada tipo florestal foram selecionados dois estratos e localidades dez unidades de amostragem em cada. A unidade de amostragem é um transecto Norte-Sul, com 200 m, que sobrepõe os outros comprimentos (100 e 150 m), e três transectos (para cada comprimento) na direção Leste-Oeste, formando a cruz, totalizando 650m. Foram medidos todos os elementos de madeira morta grossa (CWD) que cruzaram o transecto com um diâmetro ≥ 10 cm. Foi medido o diâmetro na interseção, o comprimento, a largura perpendicular, o elemento foi classificado de acordo com a classe de decomposição e, quando possível, a espécie foi identificada. De cada elemento foi retirado um disco de amostra na interseção, este foi fotografado para o cálculo da área oca. De cada disco foram extraídos cilindros, secos em estufa para determinação da densidade, estes foram moídos e analisados para determinação do teor de carbono (%). O volume de cada elemento foi calculado pela diferença da área seccional e área oca, multiplicada pelo comprimento; a biomassa foi calculada multiplicando o volume pela densidade; e o estoque de carbono foi calculado multiplicando a biomassa pela concentração de carbono. Os estimadores do total foram calculados por área e por hectare para o número de elementos de CWD, volume, biomassa e estoque de carbono; foram calculados por estrato, e combinados para a população. Os dados dos 650m de cada unidade foram utilizados para calcular as médias da densidade da madeira e concentração de carbono por classe de decomposição e, para construção de modelos lineares e não-lineares. Para a SSF, o design mais preciso foi transectos em forma de cruz, com 200 m. Para CSS foram selecionados transectos em cruz, com 150 m. As áreas apresentaram valores de biomassa 1.3-6.7 Mg/ha para CSS e SSF, respectivamente. Em ambos tipos florestais foi encontrado que à medida que a classe de decomposição aumenta (mais fresco para mais podre), a densidade da madeira diminui, seguindo os mesmos padrões encontrados na literatura. A concentração de carbono não apresentou mudança dentro das classes de decomposição. Modelos não-lineares com diâmetro e comprimento como variáveis preditoras provaram ser uma ferramenta eficiente para prever a biomassa e carbono de CWD. Como esperado, os dados de biomassa apresentaram heterocedasticidade, mitigada pela modelagem da variância dos resíduos com uma função de potência. A adição da classe de decomposição como variável indicadora também mostrou melhora nos modelos.

Palavras-chave: Madeira morta; Amostragem pela linha interceptadora; Biomassa; Carbono; Densidade da madeira; Equação alométrica; Floresta Estacional Semidecidual; Cerrado *sensu-stricto*

ABSTRACT

Estimation of volume, biomass, and carbon of coarse woody debris in native forests in São Paulo State, Brazil

The objective of this study was to test the line intersect sampling (LIS) methodology using the design-based inference with stratified systematic sampling and two transect shapes: a straight line and a cross shape, both with three different lengths (100, 150, and 200 m) to estimate the number of coarse woody debris (CWD) elements, their volume, biomass and carbon stocks; by decay class; furthermore, we fit and select CWD biomass and carbon models. The study area includes two types of native forest in the State of São Paulo, Brazil: a Seasonal Semi-deciduous Forest (SSF) and a Cerrado *sensu-stricto* (CSS). Two strata were chosen in each area, and in each stratum ten sampling units were installed according to a systematic sampling protocol. Each sampling unit had one North-South line of 200 m superimposed over the other lengths (100 and 150 m) and three lines (one for each length) in the East-West direction forming the cross shapes, for a total 650 m per sampling unit. All CWD elements with a diameter ≥ 10 cm that crossed the transect were tallied. For each element, the diameter, length, perpendicular width, decay class, and (when possible) species was recorded. Disc samples were taken from each element, from which cylinder samples were extracted then oven dried to determine density. These cylinders were milled and analyzed to determine carbon content (%). The volume of each element was calculated by taking the difference between cross-sectional area and any hollowed area, then multiplying by the element's length. Biomass was calculated by multiplying volume values by density values, and carbon stocks were calculated by multiplying biomass by the carbon percentage factors computed via lab analysis. Total estimators were calculated by area and per hectare for the number of CWD elements, their volume, biomass, and carbon stocks. These estimators were calculated by stratum then combined across the entire sample population. The data from each sampling unit was also used to calculate the wood density and carbon concentration by decay class, as well as to fit linear and nonlinear models. For the SSF area, the most accurate transect design was the 200 m cross shaped; and for the CSS area was selected the 150 m cross shape. Both areas showed lower biomass values (1.3 and 6.7 Mg/ha for the CSS and SSF areas, respectively) than other studies in the Amazon Forest where CWD research has been conducted. In both areas, as the decay class increased (from least to most rotten material), wood density decreased, which follows the same pattern as other literature. Carbon concentration barely changed within decay classes. Using a conversion constant of 50%, similar carbon stock results were obtained. Nonlinear models (using diameter and length as predictor variables) proved an efficient tool for predicting CWD biomass at an element level. As expected, biomass data exhibited heteroscedasticity, which was mitigated by modeling the variance of the residuals with a power function of the combined variable. Adding decay class as an indicator variable also resulted in model improvement.

Keywords: Coarse woody debris; Line intersect sampling; Biomass; Carbon; Wood density; Allometric equation; Seasonal Semi-deciduous Forest; Cerrado *sensu-stricto*

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1 GENERAL INTRODUCTION

Brazil, with at least 10% of the total number of species on the planet, is one of the most biologically diverse countries. It has the most varied flora in the world, with 50-56 thousand species of higher plants described. Brazil's diversity is distributed in biomes such as the Amazon Rainforest, the largest remaining rainforest; the Cerrado (Brazilian savanna) biome, which includes alpine pastures and is the largest area of savannah in a single country; the Atlantic Forest, extending South to Northeast and one of the most important repositories of biological diversity; the Caatinga with vast semiarid extensions, including deciduous forests and remnants of rainforests; the Pantanal, an area that represents the most significant wetland ecosystem known; and coastal and marine biomes that hold a wide variety of ecosystems including coral reefs, dunes, wetlands, lagoons, estuaries and mangroves (BRASIL, 1998).

The Cerrado and Atlantic Forest biomes are considered hotspots, areas with high biodiversity that are endangered at the highest degree. They are priority areas for biodiversity conservation (MYERS *et al.*, 2000). In São Paulo State, southeastern Brazil, the most industrialized and urbanized state in the country, these two ecosystems predominate. The Cerrado, rich with unique vegetation types, once covered roughly 14% of the state territory, and it currently exists only in isolated fragments accounting for less than 7% of its original area. The Cerrado in São Paulo State is divided into two major groups: the grassland - dominated central to northeastern areas and, in the western region of the state, the "cerradões" (forest phase of the Cerrado) predominate (DURIGAN *et al.*, 2003). Remnants of the Atlantic Forest in Brazil account for 8% of its original area; its associated ecosystems include Dense Rain Forest, Araucaria Forest, Rain Forest Open, Semi-deciduous Forest, Deciduous Forest, Mangroves, Salt Marshes (VELOSO *et al.*, 1991). While the São Paulo State originally had approximately 82% of its territory covered by the Atlantic Forest, its current rate of cover is 8.3% (RBMA, 1997).

These numbers show the precarious position of the vegetation remaining in São Paulo State indicating the need for greater incentives for conservation and preservation projects; since they are areas of native vegetative cover that still provide important ecosystem services such as protection of water sources, slope retention, climate regulation, biodiversity maintenance and carbon storage. Current large-scale deforestation has consequences for biodiversity, rainfall reduction, soil erosion and degradation, and increasing global climate change. Since 1850, deforestation has contributed 45% of increase of the carbon dioxide and other greenhouse gas (GHG) emissions into the atmosphere. Carbon dioxide (CO_2) is credited as the main GHG, therefore the increasing concentration of this gas in the atmosphere has caused great concern (BROWN *et al.*, 1989). Efficient forest management, restoration and preservation of forests play an important role in reducing GHG emissions (MALHI *et al.*, 2002).

Studies of carbon quantification in forest compartments are relevant because in addition to contributing to the scientific community, they contribute to the accurate valuation of carbon associated with environmental services. Studies show that the total carbon stocked in the biosphere can amount to roughly 2,650 gigatons, including organisms, organic debris and sediments (RICKLEFS, 2003). Plants store atmospheric carbon in biomass, which is the difference between production from photosynthesis and consumption for respiration, usually expressed as dry weight per unit area. Biomass change can be considered a measurement parameter of changes in the forest structure (BROWN, 1997). Estimating forest biomass is extremely important for assessing climatic effect, since forest biomass is a component of the carbon stocks used to quantify the CO_2 released into the atmosphere, in this case due to forest degradation (HIGUCHI *et al.*, 1998). Carbon sequestration in forests is an environmental service highly valued by markets moving the global economy, especially for countries that need to fulfill their commitments under the Kyoto Protocol (ICB, 2012).

In a forested environment, there are various forms of biomass accumulation: above ground (trunk, branches and leaves), below ground (tree roots), litter, dead wood and soil carbon (BROWN,

1997, 2002; IPCC, 2006). Most research is focused on estimating the living biomass, which focuses on aboveground structures of trees, because above-ground arboreal carbon stocks account for the largest fraction of the total biomass. The quantification methods are simple and do not usually represent many logistical problems. However, they do not involve other structures in forest biomass estimation, thereby underestimating the magnitude of carbon sequestration in the environment (BROWN, 1997).

Dead wood is considered an important component of the forest ecosystem. It contains carbon in coarse woody debris (CWD), coarse roots, standing trees and other dead material not included in litter or soil (IPCC, 2006). Woody debris reduces soil erosion, acting as a reservoir of nutrients, energy and water, influencing the development of forest cover. It is a habitat for microbial life, vertebrates and invertebrates, storing large amounts of carbon (HARMON *et al.*, 1994; HARMON and SEXTON, 1996; KELLER *et al.*, 2004). For the Intergovernmental Panel on Climate Change (IPCC, 2006) woody debris includes wood lying on the surface, dead roots and branches with diameter larger or equal to 10 cm. Dead biomass, also known as necromass when found above the ground, can be divided into fine and coarse according to classes of diameter. RICE *et al.* (2004), in the Forest of Tapajós, Pará State, Brazil, showed that the biomass of CWD accounted for 25% of carbon stock above ground. Given its importance to the environment, studies to quantify CWD are highly relevant ecosystems monitoring (GALVÃO FILHO, 2010).

Studies on the dynamics for the quantification of dead wood have many practical limitations, mainly in terms of field measurements, because the amount of dead wood is highly variable, both in managed and unmanaged sites (IPCC, 2006). The amount of dead wood depends on the time of measurement since the last disturbance, the type of the last disturbance, losses during the disturbance, mortality at the time of the disturbance, natural mortality. All these factors affect the total amount of dead wood (HARMON *et al.*, 1986). A common method for quantifying CWD is line intersect sampling (LIS), where measurement of pieces of downed dead wood that intersect the sampling transect are collected. The method is direct, simple to implement in the field and sometimes with lower cost than conventional surveys. With LIS it is possible to estimate CWD volume and biomass using a design-based approach, in which the population is considered fixed while the randomness enters into the inference procedure only by the probabilistic inclusion of pieces into the sample following the sampling design (KAISER, 1983; GREGOIRE and VALENTINE, 2008).

Few studies have been conducted on necromass, especially in the Atlantic Forest and Cerrado. From among the studies conducted in Brazil, the current work highlight several developed in the Amazon Rainforest (BARBOSA, 2001; SALESKA *et al.*, 2003; CHAMBERS *et al.*, 2000; KELLER *et al.*, 2004; RICE *et al.*, 2004; CHAO *et al.*, 2009; CRUZ FILHO and SILVA, 2009; GALVÃO FILHO, 2010). In the Brazilian Atlantic Forest, the current study referred to an estimate of dead wood stock along a longitudinal gradient (VEIGA, 2010); a study in a Araucaria Forest and Seasonal Semi-deciduous Forest (SANQUETTA *et al.*, 2014); a sampling study in a Araucaria Forest (DEUS, 2015); stocks of necromass in Seasonal Semi-deciduous Forest fragments (SILVA, 2013); study of carbon stocks in the coastal Atlantic Forest (VIEIRA *et al.*, 2011); and a comparison between necromass stocks in Cerrado and in a Dense Ombrophilous Forest in São Paulo State (LUCCA, 2011). More studies are needed in different ecosystems for a better understanding of production and necromass decomposition to develop the most accurate estimation models for biomass and carbon stocks (EATON and LAWRENCE, 2006).

1.1 Objectives

1.1.1 General Objective

This research used the theory of the line intersect sampling (LIS) method to develop a methodology to quantify the volume of coarse woody debris (CWD), its biomass and its carbon stock. The

research tested three lengths and two shapes of transects, with a stratified systematic sampling technique, in two vegetation types in São Paulo State: Cerrado *sensu-stricto* and Seasonal Semi-deciduous Forest.

1.1.2 Specific objectives

In each study area:

- CWD was quantified, using stratified systematic sampling in two transect shapes: straight line and cross shape;
- three different lengths of transect were tested: 100, 150 and 200 meters;
- calculate the estimators of volume, biomass and carbon of CWD through the theory of LIS using the design-based inference;
- the wood density and carbon concentration of the CWD was determined in the different classes of decay;
- models were fitted and selected for prediction of the biomass and carbon of CWD within the decay classes.

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2 LITERATURE REVIEW

2.1 Climate changes

According to the first Article of the United Nations Framework Convention on Climate Change, climate change is the change attributed directly or indirectly to human activity that alters the composition of the atmosphere (UNITED NATIONS, 1992). The Intergovernmental Panel on Climate Change (IPCC) definition differs from the United Nations definition. The IPCC defines climate change as changes in the mean and/or variability of climatic properties changes that persist for decades or longer; it refers to any change in climate over time, whether due to natural variability or the result of human activity (IPCC, 2007).

Climate change is a still-debated topic that affects political and economic affairs world-wide. While the debate continues, the demand for scientific and technological development to mitigate the effects of climate change continues to increase. Tropical forests are considered the richest environments for carbon content and biodiversity on Earth (FAO, 2010): there conservation, management and restoration have been identified as alternatives for the reduction of greenhouse gases (GHGs) since they retain carbon through bioaccumulation in plant tissues (NUNES, 2011). Deforestation is one of the activities that most contributes to global warming by releasing GHG, second only to the energy sector; thus, Brazil's capacity to reduce GHG emissions is a valuable resource to mitigate global warming (FEARNSIDE, 2008).

The main GHGs are carbon dioxide (CO_2), derived from the burning of fossil fuels and deforestation, as well as methane (CH_4) and nitrous oxide (N_2O), from agricultural activities (CGEE, 2010). It is estimated that the average global temperatures will increase between 1.1 and 6.4° C by the end of the century (IPCC, 2007). The Amazon contains more stored carbon than a decade of global CO_2 emissions induced by human activity (60-80 billion tons). This carbon continues to be gradually released into the atmosphere through deforestation (CARVALHO *et al.*, 2004).

Problems related to climate change began to be discussed seriously at the international level from the Rio Conference in 1992. However, before that there were already initiatives, as in a 1971 study of human impact on climate, organized by the Sweden Academy of Sciences, in order to influence the UN conference. In 1972 the United Nations Environment Programme (UNEP) was created, and in 1988 the Intergovernmental Panel on Climate Change (IPCC) was created, the latter in order to provide a scientific view of climate change.

In 1992, in addition to the United Nations Conference on Environment and Development (Rio-92), which included signed several protocols and treaties, such as Agenda 21, there was the creation of the United Nations Framework Convention on Climate Change (UNFCCC), which in 1994 began its mission to stabilize concentrations of greenhouse gases in the atmosphere, reducing the anthropic interference. Also at the 1992 Convention, the principle of "common but differentiated responsibilities" was established; that is, all countries should reduce their emissions of GHGs but the effort should be greater for those who have emitted more throughout history.

In 1997 the COP-3, the third Conference of Parties, was held. This annual meeting of the Convention's signatory countries was where the Kyoto Protocol was established. The Protocol, ratified only in 2005, sets GHG reduction targets for countries that signed the agreement. The period of the Protocol was set from 2008 to 2012. To help countries meet their emissions targets and encourage the private sector and developing countries to contribute to emission reduction efforts, the Protocol included three mechanisms: International Emissions Trading (IET), the Clean Development Mechanism (CDM) and Joint Implementation (JI) (UNFCCC, 2016b).

The Kyoto Protocol requires that countries reduce their emissions of GHGs. To help countries reach their emission targets and encourage the private sector as well as developing countries to reduce

emissions. The CDM stimulates sustainable development and emission reductions allowing a country with a reduction commitment through the Protocol to implement emission reduction projects in developing countries, permitting these Projects to receive credits of Certified Emission Reductions (CER_s), each credit equivalent to one ton of CO_2 . CER_s can be traded, sold and used by industrialized countries to meet their reduction targets, since the credit reductions have economic values (UNFCCC, 2016b).

By 2012, the CDM had registered more than five thousand projects, reducing over a billion tons of CO_2 equivalent since 2004 (UNFCCC, 2012). The number of projects in Brazil had been increasing (4%), however, it was still far from the proportion from China (49%) and India (21%). Brazil has abundant natural resources with great potential for the implementation of CDM projects, mainly in forests. In 2015, projects from China accounted for 60% of projects registered, while from Brazil, the number accounted for 5%. The total credits of CER_s expected from registered projects in Brazil is significantly far below the ideal (UNFCCC, 2016a).

In the 2008 COP-14 conference, held in Poland, the implementation of Reducing Emissions from Deforestation and Forest Degradation (REDD) was discussed, to create a financial value for the carbon stored in forests, making it possible to offer incentives for developing countries to reduce emissions from forested lands and invest in low-carbon transitions to sustainable development (UN-REDD PROGRAMME, 2016). In 2009, in Copenhagen, COP-15 encouraged REDD + actions, Deforestation Emission Reduction and Forest Degradation with an addendum in conservation initiatives, forest management and enhancement of carbon stocks in forests. In order to quantify GHG emissions resulting from the reduction of deforestation and forest degradation, the concept of REDD (Reducing Emissions from Deforestation and Forest Degradation) was created to attribute a financial value to the carbon stocked in forests, offering incentives to developing countries to reduce emissions from forested lands and invest in low carbon emission generating options for sustainable development. REDD + goes beyond deforestation and forest degradation and includes the roles of conservation, sustainable management of forests and enhancement of forest carbon stocks (UN-REDD PROGRAMME, 2016).

In 2012, the COP-18 established the second period of the Kyoto Protocol, effective in 2013, with the completion forecast for 2020. At COP-19, in 2013, it was agreed that all members would start cutting emissions as soon as possible, preferably by the first quarter of 2015, and the Warsaw Mechanism was also proposed. At the 2014 Conference, the main objective was to reduce emissions of greenhouse gas effect such that the global temperature increase is limited to 2°C above current levels by the end of this century. In December of 2015 at the COP-21 in Paris the parties developed an agreement that they will “pursue efforts to” limit the temperature increase to 1.5°C .

The CDM included in the Kyoto Protocol, allows emission reduction projects implemented in developing countries to sell emission reduction certificates for project components with emission reduction targets within Protocol (CARVALHO *et al.*, 2004; UNFCCC, 2016b). The claims of Certified Emission Reduction (CERs), with each credit equivalent to a carbon dioxide tonne, and can be traded, sold and used by developed countries as part of meeting reduction targets, since these credits have economic values (UNFCCC, 2016b). There are several design options to be considered under the CDM, both in forest industries and energy, as well as in the production of combined energy, industrial applications and changes in land use; options include tree plantations and forest regeneration. In addition, the Protocol encourages the parties to protect and enhance sinks and reserves, promoting sustainable forest management practices, afforestation and reforestation (CARVALHO *et al.*, 2004).

Research published in 2009 states that the rise in temperature cannot exceed 2°C because the consequences could be severe. For reducing the emission of greenhouse gases in the atmosphere, researchers suggest mitigating deforestation and recovering degraded areas. The forestry sector accounts for 72% of emission reduction potential in Brazil by 2030 (McKINSEY & COMPANY, 2009). Creating incentives for forest conservation and reduction of deforestation may be a unique opportunity for both

the conservation of forests and the mitigation of climate change (CARVALHO *et al.*, 2004). The future of the Kyoto Protocol is uncertain, but the contribution of deforestation of tropical forests to global climate change is not. There is still time for scientists and politics scholars to take advantage of what is certainly among the greatest opportunities for today's global environment: international trade in carbon emissions for the protection of tropical forests, preventing reduction of these forests to insignificance (SANTILLI *et al.*, 2005).

The issue of climate change is part of the international political and economic reality, demanding scientific development and technological innovation. The possibility of using the CDM is increasingly attracting the attention of the business sector. In addition to the economic opportunities of the CDM, risks associated with climate changes induce an increased commitment and sense of responsibility with resulting mitigation activity and adoption of appropriate production processes and consumption patterns. To reduce climate change effects, the combination of three actions is needed: inertia, adaptation and mitigation. These actions involve cost and commitments that should be discussed and shared, a reality that creates the need for international treaties to manage the global community (CGEE, 2010).

2.2 Dead woody debris and the carbon sequestration

Forest ecosystems are more than just a set of trees. They are flora associations (trees, shrubs, herbs, ferns and mosses), wildlife (mammals, reptiles, birds, amphibians), other life (arthropods and other invertebrates, fungi, soil microbes) and abiotic entities (decomposing organic matter, mineral soil and water), coexisting in a complex unit. The components comprising forest dead material are dead wood, litter and fine organic matter, as well as harvest piles; shrubs and herbaceous material are included (WOODALL and WILLIAMS, 2005). Dead wood is an important component of the forest ecosystem because it contains carbon in coarse and fine woody debris, coarse roots, standing trees and other dead material not included in the litter or soil organic matter (IPCC, 2006). Dead woody debris (DWD) reduces soil erosion; is a reservoir of nutrients, energy and water; influences the development of forest; provides habitats for microbial life, vertebrate and invertebrate animals; and stores large amounts of carbon (HARMON *et al.*, 1994; HARMON and SEXTON, 1996; KELLER *et al.*, 2004; USDA, 2011).

For ecologists, the biggest distinctions among DWD classes are based on the size (length and diameter) and position (standing or downed). HARMON and SEXTON (1996) asserted that there was no agreement on common terms to describe the DWD and that this creates a lot of confusion and barriers for comparison. The most important distinction is between coarse woody debris (CWD) and fine woody debris (FWD), the limits of which depend on the ecosystem being studied. For most forests it is recommended that the FWD have maximum diameter of 10 cm. In general, dead wood includes wood lying on the surface, dead roots, stumps with diameters larger than or equal to 10 cm (or any other diameter used for the ecosystem or by the country). FAO (2010) defines dead wood as all dead wood biomass standing or lying on the floor or ground, not contained in the litter. For the IPCC (2006), dead wood includes downed wood on the surface, dead roots and branches with diameter larger or equal to 10 cm.

While the minimum diameter of 10 cm is commonly used to distinguish CWD from FWD, this threshold diameter may vary not only according to ecosystem but also according to the research objectives. In some cases, CWD elements are defined based on the minimum diameter and the length (> 10 cm in diameter and > 1.5 m length, for example) of the element (MARSHALL *et al.*, 2000, 2003). For WOODALL and WILLIAMS (2005), CWD includes downed elements of wood of minimum 7.6 cm in diameter and minimum 0.9 m in length. LARJAVAARA and MULLER-LANDAU (2009) define CWD elements with diameter of at least 20 cm at the intersection point with the sample line and FWD with a diameter between 2 and 19.9 cm. Some authors define CWD as dead material, in different degrees of

decomposition, located above ground, with a diameter greater than 7.5 cm (PBC, 2010; VANDERHOOF and WILLIAMS, 2014; BCMOF, 2016). KELLER *et al.* (2004) state that the CWD is all dead material lying on the forest floor with a diameter greater than 2 cm, found both in unexplored and logged areas. Although some studies consider CWD elements with diameter greater than 2 cm, the inventory methods differ for elements with a diameter greater than 10 cm (KELLER *et al.*, 2004).

DWD includes all forms of dead woody material, above or below ground. The CWD is distinguished into standing elements ($> 45^\circ$) and downed elements ($< 45^\circ$). DWD number and size class are highly correlated, probably because as the tree dies the proportion of the size classes of dead trees reflect the proportion of size classes of live trees (LEOPOLD, 1971). Thus, it is common to find that FWD are fewer than 20% of CWD total. A strong correlation also exists between DWD element number above the ground and dead coarse roots. The weakest DWD correlation is between standing or suspended trees and downed material: dead standing materials may contribute 2% to 98% of total DWD (HARMON *et al.*, 1986).

The ratio between DWD standing and downed depends on factors that include mortality, forest age and tree species. The ingrowth of DWD is mainly associated with mortality of trees (HARMON and SEXTON, 1996). Some causes of tree mortality may be obvious, such as wind and fire. Tree mortality classes are categorized as dead standing, uprooted and broken (CHAO and PHILLIPS, 2005). GALE and BARFOD (1999) suggest that different positions at dying are caused by different factors: if the tree died standing, death is more influenced by biotic agents; the breaking of trees is caused in part by physical and partly by biotic agents; while uprooted trees with the roots are caused by extrinsic physical agents. The dimensions of the causes of mortality include the intrinsic factors of the tree itself, and biotic and abiotic extrinsic factors. Since different causes of death affect the dynamics of the forest, it is important to determine the dominant cause in an area and identify the factors that control the mortality patterns in the forest (CHAO and PHILLIPS, 2005).

CWD serves as habitat for numerous species of plants and fauna, ranging from large mammals to invertebrates, as well as fungi, bryophytes and lichens (HARMON *et al.*, 1986; WOODALL and WILLIAMS, 2005). The dead woody material are important for habitat assessment and wildlife conservation (WOODALL and WILLIAMS, 2005). The flora are favored by the humidity, shade and nutrients provided by CWD to establish the regeneration process (HARMON *et al.*, 1986). The amount, structure and dynamics of coarse woody debris in a forest influence nutrient cycling and productivity, stabilize the soil, contribute to global carbon storage and provide biological inheritance; all of these are structural and functional relationships between the present forests and the forests of the future (HARMON and CHEN, 1991).

Generally CWD is divided into five classes of decomposition. This attribute is not necessary for determining the volume or density; the necessity of its determination depends on the purpose of the study. Classes are defined both by physical characteristics (presence of leaves, twigs, branches, bark percentage of coverage, form) and by biological indicators (moss cover, root invasion). The evaluation of the decomposition class can be made at the intersection point or an average overall condition of the element, with the chosen method used throughout a study (MARSHALL *et al.*, 2000).

FWD, typically with a diameter less than 10 cm, is a very important, yet little-studied, part of the forest ecosystem (WOODALL and LIKNES, 2008). Formed by dead branches – free or attached to stalks, woody roots and small woody stems, this material stocks carbon and nutrients (WEI *et al.*, 1997), providing a source of food and habitat for vertebrates, invertebrates and fungi (NORDÉN *et al.*, 2004).

Woody debris inventory can be used to address three issues: 1) classification of individual plots into various categories, for example to determine whether the amount of CWD in a plot meets the requirements for suitable habitat; 2) values estimation of plots per hectare, mapping values to determine spatial patterns of woody debris or monitoring areas over time to indicate changes in woody debris

resources; and 3) the total population estimate, which can be used to assess the amount of carbon sequestered in woody debris (WOODALL and WILLIAMS, 2005). Woody debris is an important component in carbon sequestration occurring in the forests, and its quantification is essential for efficient forest management: understanding carbon location is critical to understanding the dynamics of the greenhouse effect and climate change (SMITH, 2004).

Carbon pools can be estimated jointly, combining the inventories of soils and woody debris. The woody debris inventories provide the data for the carbon estimation of CWD and FWD, and soils inventories provide carbon estimates of litter and organic matter (O'NEILL *et al.*, 2003). HARMON *et al.* (2004) found that FWD stocks 4.1% of carbon in a forest; the authors state that despite the small fraction of carbon stored in the FWD, this compartment is important for the carbon cycle in forests. Small stocks of FWD are caused in part by the high rate of decomposition in relation to CWD (HARMON *et al.*, 1995).

Downed dead wood may comprise a substantial fraction of a forest's carbon, in some places even more than carbon sequestered in the living trees. In tropical forests the amount of dead wood is not very great (10 to 15% of the biomass above ground) because this material decomposes much faster compared to areas in western North America, where large logs can remain in the soil for decades, with huge volumes of dead wood accumulating (CONDIT, 2008). Playing an important role in the carbon cycle, the world's forests stock more than 650 billion tons of carbon, 44% in biomass, 11% in dead wood and litter and 45% in the soil. The total carbon stored in dead wood and litter in 2010 was estimated at 72 billion tonnes or 17.8 tonnes per hectare. However, data regarding carbon stock in dead wood and litter are still very vague (FAO, 2010).

2.3 Estimates of biomass and carbon

The IPCC (2006) defines biomass as live material of plant and animal origin, both above and below ground, usually expressed in dry weight. Forest biomass is an important measure of ecosystem productivity and can be used to assess forest energy potential and the role of forests in the carbon cycle. Closely related, the change in biomass is an important feature of the forest ecosystem and has been part of global forest resource assessments since 1990 (FAO, 2010).

The estimate of biomass is directly linked to carbon stocks, used to quantify the CO_2 released into the atmosphere during forest degradation processes (HIGUCHI *et al.*, 1998). Vegetation stocks atmospheric carbon in biomass, which is the difference between production by photosynthesis and consumption by respiration, usually expressed as dry weight per unit area. Change in biomass may be considered a measure of changes in the forest structure (BROWN, 1997). The product of biomass and carbon concentration estimates the carbon stock of vegetation (HIGUCHI *et al.*, 2004). The more accurate the carbon stock estimate, the more accurate are the quantifications of carbon stored and carbon released into the atmosphere by forests (SANQUETTA, 2002).

Most research is focused on the estimation of living biomass, limited to the biomass of arboreal structures above ground: because these types of carbon pools represent the largest fraction of total biomass, and quantification methods are simple and usually do not pose many logistical problems. However, biomass estimations not including other forest structures result in an underestimation of the amount of the environmental carbon sequestration (BROWN, 1997). In a forest environment there are various forms of tree biomass accumulation: above the ground, the aerial part of the tree (trunk, branches and leaves); below ground, the roots of trees (less than 2 mm); litter; dead wood and carbon in the soil (BROWN, 1997, 2002; IPCC, 2006).

Forests stock over 45% of carbon and can sequester large amounts of carbon annually (Bonan, 2008). It has been estimated that globally forests have 600 Gt (gigatonnes) of biomass above and below ground, of which 67 Gt, or 16.6 tons per hectare, are of dead wood (FAO, 2010). According to RICE *et al.*

(2004) in the Tapajós Forest-Pará dead woody debris biomass represented 25% of the stock of carbon above ground. Dead wood biomass stocks decreased about 3 Gt over the period 1990-2010, mainly due to reduction of forest areas (FAO, 2010).

Quantification of carbon can increase understanding of climate change and the role of forest carbon dynamics in this scenario. The dead woody material increased the accuracy in carbon estimates (WOODALL and WILLIAMS, 2005). With so much functionality in the environment, DWD study is highly relevant in monitoring ecosystems (GALVÃO FILHO, 2010).

2.4 Sampling

Sampling quantifies information about the entire population by observing only a part, a sample, of the population. Inferences obtained from the sample for the population are reliable if the sample is a true representation of the population of interest. Compared with measuring a full population, often impossible even if it were desired, the sampling process reduces costs, reduces the time spent, is applicable in various situations, has greater accuracy and provide more reliable information (AVERY and BURKHART, 1983).

When planning a sample, the sample design, as well as sample size, the shape and size of the sampling unit are considered and pre-planned (AVERY and BURKHART, 1983). The sampling design determines the sampling method, referring to the layout and data collection approach relating to the sampling unit, such as: fixed area method, Bitterlich method, line intersect method, among numerous others.

The set of sampling units is part of the sampling process, which also includes the frequency with which sampling is performed (PÉLLICO NETO and BRENA, 1997). Sampling processes can be classified into random, where the sampling units are selected completely random from the population; cluster: according to QUEIROZ *et al.* (2011) cluster sampling came from the knowledge that random units distributed in a population could be grouped in order to reduce costs in the field work, especially when the population is difficult to access; and systematic, in which occurs the randomization of the first sampling unit only, then the next units will be selected follow a predetermined system; and cluster.

2.5 Line Intersect Sampling

In many recent dead woody debris inventories the method of line intersect sampling (LIS) has been used. In the method the sampling units are transects, in which the downed dead woody debris elements that are intersected by the transect are measured. WARREN and OLSEN (1964) note that an advantage of this sampling technique is that the total volume of a sampled area can be estimated by measuring only the diameter or cross-sectional area of a woody residue element at the intersection point with the transect. Before LIS was introduced, dead woody debris was sampled through the census of all elements in a given area or through the use of strip samples (WOODALL and WILLIAMS, 2005). In some literature on LIS, the elements of the population are termed *particles* or *pieces*. In this study the work of GREGOIRE and VALENTINE (2008, pp. 279-325) is followed, using the neutral term, *element*.

Some aspects of the LIS method are related to the *The Buffon Needle Problem*, one of the oldest problems in dealing with the theory of geometric probability, introduced and solved by Buffon in 1777 (SINIKSARAN, 2008). In the problem, a needle length l is dropped over a flat surface labeled with parallel lines spaced at distance d apart. The question posed by Buffon was to determine the probability that a randomly dropped needle crossed one of the marked lines. The solution is $p = 2l/\pi d$. If the needle is thrown m times and $t_{ks} = 1$ to s^{th} dropped needle intersects a line, otherwise $t_s = 0$, then t_s is a

Bernoulli random variable, with success probability of p (PERLMAN and WICHURA, 1975; GREGOIRE and VALENTINE, 2008; SINIKSARAN, 2008).

CANFIELD (1941) introduced the concept of LIS to estimate the density of a range of vegetation, incorporating the random location of the transect and taking the ratio of the transect line intersected by vegetation. The result is an unbiased estimator of canopy cover, i.e., the proportion of the area coverage by vegetation to the area of the region of interest. The author did not show that this estimator was unbiased. Canfield called the method the “line-interception method.” He also discussed design issues such as how many lines are needed, what the length of these lines should be and whether the area of interest should be stratified or not. KAISER (1983) proved the design-based unbiasedness of this estimator conditionally on transect orientation.

MCINTYRE (1953) explored the possibility of using the data on intercept lengths to estimate density, i.e., the ratio of the number of elements to the area of the interest region; the author was able to apply this for populations with elements of known form. MATÉRN (1964) used the methodology for estimating the total length of roads. LUCAS and SEBER (1977) proved unbiased estimators of density and area coverage to elements with arbitrary shapes and located when the transect is placed randomly. The area coverage estimator was the same presented by CANFIELD (1941). EBERHARDT (1978) reviewed three transects methods: line intersect sampling, line transect sampling and strip transect sampling. He used an example of LIS to estimate the number of den sites of local prairie dog in a big city. It is important to note that, despite the similarity in name, LIS is not the same as line transect sampling, as the latter is used to sample wildlife and other mobile populations (GREGOIRE and VALENTINE, 2008). SEBER and PEMBERTON (1979) studied LIS to estimate aspects of plant cuticles in fecal and rumen samples on microscope slides.

The term “line intersect sampling” was introduced by WARREN and OLSEN (1964), who were also the originators of using the method in forested environments as a way to quickly estimate the volume of waste produced in logging during forestry operations; they extended LIS to forestry applications by assuming that the elements have the form of needles. VAN WAGNER (1968) presented the bias estimator of WARREN and OLSEN (1964) under a certain set of assumptions: one of them that the elements are placed and oriented uniformly and randomly in the area. Based on similar assumptions, DE VRIES (1973) obtained unbiased estimators of total of arbitrary elements and considered variances and estimation of variance. Again under the assumption of random location of the elements, DE VRIES and VAN EIJNSBERGEN (1973) showed estimators of the total of arbitrary elements allowing shapes of arbitrary elements and comparing to the case where the parts are circular arcs. BROWN and ROUSSOPOULOS (1974) introduced a method for eliminating the influence of inclination angles of non-horizontal elements. PICKFORD and HAZARD (1978) conducted a series of simulated LIS studies on forest residue.

A point of confusion concerning LIS is that the properties of the estimators may follow a design-based approach (KAISER, 1983; GREGOIRE, 1998; GREGOIRE and MONKEVICH, 1994; GREGOIRE and VALENTINE, 2003; WILLIAMS and GOVE, 2003; GREGOIRE and VALENTINE, 2008) or a model-based approach (BELL *et al.*, 1996; BROWN, 1974; DE VRIES, 1973, 1986; VAN WAGNER, 1982, 1968; WARREN and OLSEN, 1964). GREGOIRE (1998) believes that the confusion over model-based inference has been supported in part by an indiscriminate use of terms “model-based sampling” and “model-based estimate” in contexts where model-based inference was the relevant concern.

Design-based inference is the classic detailed sampling by COCHRAN (1977) and more fully explicated in SÄRNDAL *et al.* (1992), in which the properties of an estimator are deduced from their distribution under a stipulated sample design. In design-based inference, the population is considered as fixed while the sample is considered a realization of a stochastic process (KAISER, 1983; GREGOIRE, 1998; AFFLECK *et al.*, 2005; GREGOIRE and VALENTINE, 2008). Under the design-based paradigm the reference distribution is a consequence of the sample design for a fixed population; under the model-based

paradigm the reference distribution is a consequence of an assumed model of the population behavior, i.e., a model serves as a basis for inferences about population parameters in the sampling context. The sample design is an accessory to the model-based inference (GREGOIRE, 1998).

The fundamental distinction between design-based and model-based methods is that the inference, not the estimator, is based on the model (GREGOIRE, 1998). How useful is it to distinguish design-based inference from model-based inference if both provide equally valid estimates? GREGOIRE (1998) indicates that choosing the best paradigm depends on the circumstances and the research objectives.

LIS can be used to estimate the volume of fine woody residues (diameter < 10 cm). In this approach elements are recorded by size class, and then volume is calculated. Volume calculation uses average diameter by elements size class and the average of incidence angle along the transect, the latter correcting distortions introduced by the non-horizontal orientation of elements (HARMON and SEXTON, 1996). Accurate counts of FWD can be conducted efficiently to about 50 elements for small and medium size classes and up to 20 elements for the large size class. After that, counts must be estimated in a systematic way (USDA, 2011). The required length of the transect for an adequate sampling is inversely related to the density of the elements (BROWN, 1974; PICKFORD and HAZARD, 1978), i.e., smaller elements require a shorter transect length than larger parts, because FWD frequency is higher than that of CWD (HARMON and SEXTON, 1996).

LIS has been promoted to estimate not only the DWD volume, but also a variety of ecosystem attributes (DE VRIES, 1973, 1974; KAISER, 1983; MARSHALL *et al.*, 2000; GREGOIRE and VALENTINE, 2008). LIS has been used, for example, to estimate the boundary length, diversity index, fuel loading, canopy and coverage area, forest gap size, root length, forestry operations residues and basal area of standing trees (GREGOIRE and VALENTINE, 2008).

2.6 Field protocol to sampling of dead woody debris

Many manuals and protocols for the inventory of dead woody debris are found in the literature. Most of these use the LIS method, varying, for example, sample design, number of sampling units, size and shape of transects, as well as size and decay classifications. This section presents a compilation of major manuals, focused only on foundations about the measurement of DWD elements. The elements that are intersected by the transect are measured only if they fit in the inventory rules for CWD and FWD presented in the manuals. This content is not meant to be comprehensive or to be used as a field manual. Not every rule presented here was used in this study.

Dead elements are considered measurable as woody debris (twigs, branches, trunks) of trees and shrubs. Dead branches attached to standing trunks are omitted. An element is considered downed dead material when it has fallen on the ground, or it is totally separated from its original source of growth. Root debris, sticks, leaves and grass are not measured (BROWN, 1974; MCRAE *et al.*, 1979; PBC, 2010; BCMOF, 2016). Fallen trees that still have green leaves are counted only if they do not have roots connected to the soil, which can keep the individual alive (PBC, 2010; BCMOF, 2016). Only elements that are above the ground are tallied; an element is no longer above the ground when it is completely buried under a layer of organic matter (forest floor) and/or mineral soil (BROWN, 1974; MCRAE *et al.*, 1979; PBC, 2010; USDA, 2011; BCMOF, 2016). If one end of the element is buried under a layer of soil or organic matter, the element ends at the point where it is no longer visible (PBC, 2010; USDA, 2011).

Some protocols measure only elements in which the intersect line crosses the central axis, and do not measure any element that has the central axis coinciding perfectly with the end of the intersect line (BROWN, 1974; MCRAE *et al.*, 1979; PBC, 2010; USDA, 2011; BCMOF, 2016). GREGOIRE and

VALENTINE (2003); AFFLECK *et al.* (2005) and GREGOIRE and VALENTINE (2008) consider an element completely intersected only if the sampling line intersects its boundary as many times as a coincident line of infinite length. In Figure 2.1 (a) the transect completely intersect the element, while in situations (b) and (c) are partial intersections. The element will only be included in the sample if happens the situations in (a) and (c). In (b) the partial intersection happens by the starting point, so in this case the element is omitted.

Figure 2.1: Intersection of a non-convex population element, U_k . In each transect, open dot symbolizes the starting point. Examples of complete intersection (a); examples of partial intersection with the starting point of the line (b) and; examples of partial intersection by the front end of the line (c). Source: Adapted from GREGOIRE and VALENTINE (2008)

Most CWD protocols measure the diameter of the elements, perpendicular to the central axis, at the point where the line intersects the element (BROWN, 1974; McRAE *et al.*, 1979; MARSHALL *et al.*, 2000, 2003; LARJAVAARA and MULLER-LANDAU, 2009; PBC, 2010; USDA, 2011; BCMOF, 2016). If the element is oval or semi-round, an equivalent diameter from the geometric mean is computed from the measurements of the short axis diameters and long axis (HARMON and SEXTON, 1996; MARSHALL *et al.*, 2000; USDA, 2011; BCMOF, 2016). Accurate measurement of the dimensions of CWD is essential for quality estimates of CWD attributes; working to minimize measurement errors some literature measures the diameter at the element larger end and at the smaller end, greater or equal to the minimum diameter considered by the relevant manual (GREGOIRE and VALENTINE, 2008; WOODALL and WILLIAMS, 2005; USDA, 2011). If the transect line crosses an element in more than one place, BROWN (1974); McRAE *et al.* (1979); MARSHALL *et al.* (2000); LARJAVAARA and MULLER-LANDAU (2009); PBC (2010); USDA (2011); BCMOF (2016) say to measure each intersection individually. For example, if an element is crossed by the sample line at two points, consider the diameter of each point and repeat the total length of the element, as two separate elements. GREGOIRE and VALENTINE (2003); AFFLECK *et al.* (2005); GREGOIRE and VALENTINE (2008) consider that forked elements should not be recorded as two different elements, they affirmed that each connected element constitutes only one element of the population.

It can also happen that more than one sample line cross the same element, MARSHALL *et al.* (2000) record this element as two different elements, independent probabilistic events. Following GREGOIRE and VALENTINE (2003, 2008) and AFFLECK *et al.* (2005) with a transect of J segments ($J > 1$), for example, L-shaped, +-shaped, or Y-shaped transects, there is the possibility that an element of the population be intersected by two or more lines. This fact imposes that the probability of an element being included in the sample from a transect with J segments differs from the element's probability of being intersected by J replicated transects.

CWD can be separated into standing material ($> 45^\circ$) and downed material ($< 45^\circ$) (HARMON and SEXTON, 1996; WOODALL and WILLIAMS, 2005; USDA, 2011; VANDERHOOF and WILLIAMS, 2014). At the point where the line intersects the element tilt angle is measured, using a clinometer placed on the element surface regardless of the inclination (PBC, 2010; BCMOF, 2016). Marshall *et al.*, 2000 discuss that an elements that is not lying on the horizontal plane has, in this plane, a smaller effective length than

its actual length. The smaller effective length reduces the probability of the element being crossed by the intersect line. If the tilt angle of the element from the horizontal is known, it can be accounted. If the tilt angle is less than 10 or 15 degrees it will have little effect on the effective length of the element (about 2 to 4% reduction) (MARSHALL *et al.*, 2000). LARJAVAARA and MULLER-LANDAU (2009) follow the rule that if the CWD element is not resting on the ground, it should not be considered. BROWN (1974) and MCRAE *et al.* (1979) guide to look above during the sampling process because can be computed fallen elements at any height; usually the viable measurement of these elements is about two meters in height but can be above it. PBC (2010) and BCMOF (2016) consider suspended elements only if they are being supported by other individuals and not themselves. The soil angle should be measured following the same procedure for the tilt angle (PBC, 2010).

In the LIS method the element length is measured along the central axis of the element, regardless of the shape of the element (MARSHALL *et al.*, 2000; MARSHALL and DAVIS, 2002; USDA, 2011). The element length is generally only considered in DWD with a diameter greater than or equal to the minimum diameter considered by the manual, usually 10 cm for CWD. If the diameter becomes smaller than the minimum, this part is not included in length measurements (MARSHALL *et al.*, 2000; MARSHALL and DAVIS, 2002).

For PBC (2010); USDA (2011) when the intersect line crosses the branches of a forked tree, each branch should be measured, if they have the minimum diameter. One of the trunk bifurcations (largest diameter) is determined to have its length measured to the end of the element, the main stem. The length of the smaller diameter branch, will be measured only until the junction with the main stem. If an element is broken but not separated into two, it should be considered as a whole element. If the element was divided into distinct elements, measures each element individually, if it be crossed by the intersect line and have the minimum diameter required (PBC, 2010; USDA, 2011; BCMOF, 2016).

For main boles with exposed roots, bole length is measured only down to the root collar (MARSHALL and DAVIS, 2002; PBC, 2010). Uprooted stumps and their exposed dead roots are considered according to the point that the intersect line cross, since they are larger than the minimum diameter (BROWN, 1974; MCRAE *et al.*, 1979; PBC, 2010; BCMOF, 2016). Undisturbed stumps must not be measured (BROWN, 1974; MCRAE *et al.*, 1979; PBC, 2010).

In some inventories the orientation of the central axis of the element is measured with the aid of a compass. The relative direction to magnetic north is measured by pointing the compass from the larger end of the element to the smaller and noting the nearest degree (LARJAVAARA and MULLER-LANDAU, 2009).

The perpendicular width is another attribute measured in certain protocols, defined by the perpendicular distance between the parallel tangents of the element (Figure 2.2) (KAISER, 1983; GREGOIRE and MONKEVICH, 1994; GREGOIRE, 1998; GREGOIRE and VALENTINE, 2003, 2008; AFFLECK *et al.*, 2005). This attribute can be measured with calipers with the double arms oriented parallel to the sample line (GREGOIRE, 1998). KAISER (1983) makes clear that if the element perpendicular width is measured, the Horvitz-Thompson estimator (HT) can be used to estimate any population attribute without making assumptions that the element is in the form of a needle, located randomly, etc.

In the case of FWD, rather than actually measuring the diameter of each element that crosses the line, the elements are counted because it would be impractical in the field to measure the diameter of hundreds of FWD elements (WOODALL and WILLIAMS, 2005). With the count it is possible to determine FWD volume using mean square diameter and size class (WOODALL and WILLIAMS, 2005; USDA, 2011). An element is included only if it is woody; neither pinecones, nor non-woody parts (e.g. petiole and rachis) of trees or bushes are included. If there is no record on a transect, a zero is entered for the count. Path lines falling over areas with high density of FWD, where the count is almost impossible, can be sub-sampled. Where rocks, logs, or other obstacles are present along the transect, any FWD elements on top

Figure 2.2: Perpendicular width (w_k) of the element U_k , the direction of the s^{th} transect line is represented by θ_s . Source: Adapted from GREGOIRE and VALENTINE (2008)

of these obstructions are included (USDA, 2011).

Fewer attributes are estimated for FWD than for CWD, usually estimating only volume and weight. The estimation of other parameters requires the measurement of the length of each element. Length measurement of FWD is impracticable in areas where a large number of elements are found. For CWD volume, weight, number of elements, suitable habitat and other attributes can be estimated: detailed measures are taken for all CWD elements that intersect with the transect (WOODALL and WILLIAMS, 2005).

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3 SAMPLING FOR THE ESTIMATION OF VOLUME, BIOMASS, AND CARBON OF COARSE WOODY DEBRIS WITHIN TWO FOREST TYPES OF THE SÃO PAULO STATE, BRAZIL

Abstract

The objective of this study was to test the line intersect sampling (LIS) methodology with a design-based inference using stratified systematic sampling and two transect types - a straight line and a cross shape - with three different lengths (100, 150, and 200 m) to estimate the number of coarse woody debris (CWD) elements, their volume, biomass, and carbon in two native forest types in the State of São Paulo, Brazil. One forest type was a Seasonal Semi-deciduous Forest (SSF) located at the Caetetus Ecological Station; the other was a Cerrado *sensu-stricto* (CSS) located in the Mogi Guaçu Biological Reserve. Two strata were designated in each area, and ten sampling units were located in each stratum. Each sampling unit had one North-South transect of 200 m superimposed atop the other lengths (100 and 150 m); three transects (one for each length) were laid in the East-West directions forming the cross shapes. All CWD elements with a diameter ≥ 10 cm that crossed the transect were tallied. For each element, the diameter, length, perpendicular width, decay class, and (when possible) species was recorded. Furthermore, disc samples were also taken from each element, and they were photographed to determine their hollowed area. From each disc cylinder, samples were extracted then oven dried to determine density. These cylinders were milled and analyzed to determine carbon content (%). The volume of each element was calculated by taking the difference between cross-sectional area and the hollowed area, multiplied by the length. Biomass was calculated by multiplying volume values by density values, and carbon stocks were calculated by multiplying biomass by the carbon factor computed via lab analysis. Sampling estimators for the number of CWD elements, volume, biomass, and carbon stocks were calculated for the total by area and by hectare. These estimators were calculated by stratum then combined across the entire sample population. For the SSF, the most accurate design was the 200 m cross shaped transect; for the CSS, the 150 m cross shaped transect was selected. These transect designs are thus recommended for this study, but more studies need to be made before concluding that they are the absolute best designs for measuring CWD in these ecosystem types. Both areas showed much lower biomass values (1.3 and 6.7 Mg/ha for the CSS and SSF areas, respectively) than other studies in the Amazon Forest where most of CWD research has been conducted. The results for the SSF were fairly similar to two other studies found in the same ecosystem type; however, only one previous study on CWD was conducted in a Cerrado ecosystem, and the results from that study showed great differences from this one. With the selected design the estimators were calculated by decay class, being the third class more representative in both forest types. As this kind of study is very important for determining the appropriate sampling design protocol for future inventories, more studies are necessary in these two types of forest to better understand how much CWD pools contribute to biomass and carbon stocks.

Keywords: Sampling estimator; Line intersect sampling; Coarse woody debris; Biomass; Carbon

3.1 Introduction

Dead wood is considered an important component of forest ecosystems as it contains carbon in the form of coarse woody debris, coarse roots, standing trees, and other dead material excluding litter and soil (IPCC, 2006). Studies that attempt to quantify dead wood have many practical limitations, mainly in terms of field measurements, because the amount of dead wood is highly variable both on managed and unmanaged sites (IPCC, 2006). WOODALL *et al.* (2009) reported that only 13% of countries conduct

necromass forest inventories with proper sampling methods and dead wood classification; most countries have only recently begun woody debris inventories and often employed low sample intensities when doing so.

Currently, a commonly accepted method for quantifying coarse woody debris is the line intersect sampling (LIS) procedure, where data on elements of downed dead wood that intersect the sampling line are collected (WARREN and OLSEN, 1964; DE VRIES, 1986; MARSHALL *et al.*, 2000, 2003; AFFLECK *et al.*, 2005; WOODALL and WILLIAMS, 2005; GREGOIRE and VALENTINE, 2008; USDA, 2011). KAISER (1983) provided a clever derivation of estimators for LIS where it is assumed (as in sampling theory) that the population is fixed; stochasticity becomes part of the design’s unbiased estimation only by the probabilistic inclusion of the elements into the sample, which is determined to the sampling scheme. Spatial arrangement, shape, dimensionality, and size characteristics associated with the elements are considered fixed, and the stochasticity is represented via the location as well as the orientation of the sampling line of common length L (KAISER, 1983; GREGOIRE, 1998; AFFLECK *et al.*, 2005; GREGOIRE and VALENTINE, 2008).

Few studies have been conducted on dead biomass, especially in the Atlantic Forest and Cerrado (Brazilian savanna woodland) areas of Brazil. Among the studies conducted in Brazil, we highlight those undertaken in the Amazon Rainforest (BARBOSA, 2001; SALESKA *et al.*, 2003; CHAMBERS *et al.*, 2000; KELLER *et al.*, 2004; RICE *et al.*, 2004; CHAO *et al.*, 2009; CRUZ FILHO and SILVA, 2009; GALVÃO FILHO, 2010). In the Brazilian Atlantic Forest, two noteworthy studies were conducted on Seasonal Semi-deciduous Forests (SILVA, 2013; SANQUETTA *et al.*, 2014), and there are other studies that were conducted within the same biome but on different physiognomy (VEIGA, 2010; VIEIRA *et al.*, 2011; DEUS, 2015; LUCCA, 2011). Only one study has been undertaken in the Cerrado ecoregion (LUCCA, 2011). More studies are needed across various ecosystems to develop a comprehensive understanding of necromass production and decomposition; furthermore, such research would allow for better estimates of biomass and carbon stocks (EATON and LAWRENCE, 2006).

This study aims to test the LIS methodology via a design-based approach for inference that uses stratified systematic sampling with two transect designs: a straight line and a cross shape, both of which were investigated with variations on design lengths (100, 150, and 200 m). Transect data were used to estimate the number of elements, volume, biomass, and carbon stocks of coarse woody debris for two types of native forests in the State of São Paulo, Brazil.

3.2 Material and methods

3.2.1 Study sites

The study was performed on two different forest ecosystem types both located in the São Paulo State. The first survey was conducted in a Seasonal Semi-deciduous Forest (SSF) in the Brazilian Atlantic Forest located at the Caetetus Ecological Station. The second survey was conducted in a Cerrado ecosystem (i.e., a tropical savanna; specifically, a Cerrado *sensu-stricto* (CSS), which is a subtype of the Cerrado ecosystem exhibiting arborescent flora) located in the Mogi Guaçu Biological Reserve (Figure 3.1).

3.2.1.1 Caetetus Ecological Station - Seasonal Semi-deciduous Forest (SSF)

The existing forest at the Caetetus Ecological Station is one of the largest remaining of Seasonal Semi-deciduous Forests in Brazil. The “semi-deciduous” qualities occur because of the seasonal cold weather and the reduced of water availability in the soil, alongside some other environmental factors. As

Figure 3.1: Location of the Caetetus Ecological Station (a Seasonal Semi-deciduous Forest - SSF) and the Mogi Guaçu Biological Reserve (a Cerrado *sensu-stricto* - CSS) in São Paulo State, Brazil

such, most of the arborescent species lose their leaves in winter to reduce water consumption and decrease their rates of growth (TABANEZ *et al.*, 2005).

Within the study area one can distinguish 12 physiognomic units (i.e., vegetation types; see Figure 3.2) differentiated by size, density, special characteristics, preservation status, and ecological conditions. In some areas, the vegetation communities feature a rich balance of life forms, with a high number of tree species at later successional stages that have been preserved well (i.e., they have well developed and unperturbed components), alongside a high density of plants across all ecosystem strata, featuring pioneer species, lianas, and a small amount of bamboo. In other areas, vegetation communities are not as evenly balanced, and there are only a small number of tree species that are sparsely populated due to high levels of disturbance that also results in a predominance of pioneer species, lianas, scattered clumps of bamboo, and an abundant as well as diverse understory (TABANEZ *et al.*, 2005).

According to the official Management Plan of the Caetetus Ecological Station, the Conservation Unit covers the cities of Galia and Alvinlândia with a total area of 2178 ha. Its elevation ranges from 520-680 m, and it is dominated by lower slopes (with less than a 6%). It is located in the northern border area of the hydrographic basin of the Paranapanema river. The climate, according to the KÖEPPEN (1948) classification, is Cwa: mesothermal dry winter with temperatures below 18° C during winter and above 22° C during summer. In the driest month, the total precipitation is only 30 mm, and the area experiences an annual precipitation from 1100 to 1700 mm (TABANEZ *et al.*, 2005).

3.2.1.2 Mogi-Guaçu Biological Reserve - Cerrado *sensu-stricto* (CSS)

The Mogi-Guaçu Biological Reserve is part of the old Campininha Farm located in the Martin Prado Junior District of the city of Mogi Guaçu, São Paulo; its geographic coordinates are: latitude 22° 18' South and longitude 47° 11' West. Its area is 470 ha, and it is a relatively flat landscape with no more than 15% slope at any point. The elevation ranges from 566 to 724 m, and the climate is mesothermal with two well defined seasons: a dry winter (between the months of April and September) and a hot summer (from October to March). The average annual rainfall is 1335 mm, and the average annual temperature is 20.5° C (SÃO PAULO, 2015).

Figure 3.2: Map of the Caetetus Ecological Station (SFF) showing vegetation types/physiognomic units. Legend code descriptions: **A** – Sparse arboreal vegetation of low stature alongside herbaceous plants and grasses with high levels of human disturbance; **B** – Dense arboreal vegetation of low stature with high anthropic interference; **C** – Well preserved arboreal vegetation of medium stature; associated with higher soil moisture; **D** – Sparse arboreal vegetation of low stature with high anthropic interference; **E** – Sparse arboreal vegetation of low stature alongside herbaceous plants and grasses; associated with higher soil moisture; **F** - Graminoid vegetation; **G** – Sparse arboreal vegetation of medium stature with remnants of selectively cut individuals; **J** – Mixed herbaceous and graminoid vegetation; exhibits a permanent soil water surplus; **L** - Arboreal vegetation of high stature that is less dense than other units; contains deciduous species that experience anthropic disturbance; **M** – Dense arboreal vegetation of high stature; well preserved forest with low quantity of deciduous species; **N** – Dense arboreal vegetation of high stature without deciduous species; well preserved with little or no human disturbance; **P** – Sparse arboreal vegetation of medium stature with a high degree of human disturbance. Source: adapted from TABANEZ *et al.* (2005)

The Reserve is divided into two plots: one designated as “Area A” and the other as “Area B”. “Area A”, which is 343.42 ha in size, is composed mostly of Cerrado ecosystems at various regeneration stages; i.e., the area is mostly savanna woodlands (with communities spanning pioneer to advanced species) and there are small patches of herbaceous vegetation near the watercourses (Figure 3.3). “Area B”, which is 126.63 ha in size, varies from cerradão to denser forest (with a majority of land area being the latter) (SÃO PAULO, 2015). The study area for this research was “Area A”.

Figure 3.3: Map of the Mogi-Guaçu Biological Reserve (CSS) with vegetation types/physiognomic units. Source: SÃO PAULO (2015)

3.2.2 Field survey

3.2.2.1 Sampling and stratification

Two transect shapes were evaluated in order to compare their respective efficiency at measuring coarse woody debris — a straight line and a cross shape. Stratified systematic sampling was applied across the study forest types. In stratified sampling, the population of N units is divided into subsets of $N_1, N_2, \dots, N_L$ units, without any overlap between subsets (COCHRAN, 1977).

The straight line transects were oriented in the North-South direction, and three transect lengths were tested: 100, 150, and 200 m (Figure 3.4). The cross shaped transect was composed of two perpendicular transects following the cardinal directions (N-S, E-W), and these transects were also tested using three line distances (100, 150, and 200 m) (Figure 3.5). The North-South straight line transects are laid coincident to one another (i.e., the 100 m line is laid, then 50 additional meters are measured two additional times to fully establish all three transect lengths, which means the 150 m and 200 m lines encompass the data from the 100 m line). After establishing the N-S lines, the E-W transects are laid at the center points of each of the three straight-line transects to form the cross shape. Figure 3.6 shows a superimposed layout of our sampling units including all transect designs and lengths.

The maps of vegetation types presented in the Management Plans of the protected areas were used as a basis for determining strata (Figure 3.3 and Figure 3.2), and two physiognomic units were selected as the strata within each of the study areas. In the Caetetus Ecological Station the strata selected were: stratum I (SSF1) - arboreal vegetation of high stature that is less dense than other units; contains deciduous species that experience anthropic disturbance; it occupies the tops of plateaus and interfluvies and is 777.8 ha in size; it was identified and mapped in the Management Plan as area “L”; and stratum II (SSF2) - dense arboreal vegetation of high stature; well preserved forest with low quantity of deciduous species; it predominates in the high escarpments and plateau edges and is 439.4 ha in size; it was identified and mapped in the Management Plan as area “M” (Figure 3.2) (TABANEZ *et al.*, 2005).

Figure 3.4: Straight line transect designs oriented in the North-South direction with three sizes tested (100, 150, and 200 m)

Figure 3.5: Cross shaped transect designs oriented in the North-South and East-West directions with three sizes tested (100, 150, and 200 m)

Figure 3.6: Superimposed straight line transects and cross shaped transects; solid lines represent straight line transects; dashed lines represent the E-W portions of the cross shaped transects (with N-S lines coincident with one another)

In the Mogi Guaçu Biological Reserve the strata selected were: stratum I (CSS1) forested savannah at an advanced successional stage, with an area of 136.5 ha; and stratum II (CSS2) forested savannah in an intermediate successional stage, with an area of 197.5 ha (Figure 3.3) (SÃO PAULO, 2015).

3.2.2.2 Location of sampling units

Ten sampling units (Figure 3.6) were installed in each stratum within both forest types; there were 650 m worth of transects at each sampling unit, totaling 6,500 m per stratum and 13,000 m per forest type. The linear distance between sampling units was 100 m. Using ArcGIS 10.2 software (ESRI, 2013), a sampling grid of point locations (300 x 300 m) was generated across the maps of each forest type. Each point constituted the center of a sampling unit (Figure 3.6), and this grid guided the distance measurement of 100 m between each sampling unit. Ten points on this grid (i.e., 10 sampling units locations) in each stratum were systematically selected taking into account suitability of access in addition to ensuring that transects did not exceed the boundaries of the study areas or stratum. Figures 3.7 and 3.9 show maps of each forest type with the grid points and the 10 points selected by stratum. Figures 3.8 and 3.10 show maps of each forest type with the sampling units (Figure 3.6) detailed in each stratum. The sampling locations, (x_s, z_s) , $s = 1, \dots, m_h$ ($m_h = 10$ for each h^{th} stratum) each served as the mid-point or the end-point of a transect of length L .

Points were located in the field with a Garmin GPS - Oregon 550. We used a professional compass (a Suunto KB-20) to ensure transect alignment along cardinal directions. A 50 m tape measure was used to confirm the transect distance and also worked as a sampling line.

3.2.2.3 Inventory Protocol

For the field survey, dead woody debris was classified into size classes according to KELLER *et al.* (2004):

Small: branches or bamboo with diameter between 2 and 5 cm;

Medium: branches or bamboo with diameter between 5 and 10 cm;

Large: woody material with diameter greater than 10 cm;

Elements belonging to the small and medium diameter classes were considered fine woody debris (FWD) and elements in the large class were considered coarse woody debris (CWD) (though this study

Figure 3.7: Caetetus Ecological Station (SSF) map with grid points (300 x 300 m) (a) and selected points (b)

deals only with CWD). The LIS method was used to sample CWD in the study areas with the sample unit being a transect line; i.e., all downed coarse dead woody that intersected the sample lines were tallied (Figure 3.11) following this protocol:

- dead woody material (e.g., branches and trunks) with diameter greater than 10 cm that was lying on the ground or inclined on an angle smaller than 45° was coded as CWD;
- dead material attached to standing trunks was omitted;
- only elements that did not have roots connected to the ground were recorded, which could somehow keep the plant alive;
- only above ground CWD elements were tallied;
- Following AFFLECK *et al.* (2005) as well as GREGOIRE and VALENTINE (2008), elements were coded as CWD based on their intersection with the transect lines (see Figures 3.12 and 3.11); an element was only included in the sample if it exhibited complete intersection with a line (Figure 3.12 (a)) or if it intersected the front end of a line (Figure 3.12 (c)). Partial intersections at the line starting points were omitted (Figure 3.12 (b));
- elements with their central axis coinciding with a transect line were omitted;
- diameters were measured with a caliper at the point where the transect line intersects the element. Two measurements were taken one parallel to the ground and the other perpendicular (Figure 3.13); if the bark was still attached to the element, the diameter measurements included it;

Figure 3.8: Caetetus Ecological Station (SSF) map with inset views of strata and sampling units. Strata sampled include: stratum SSF1 (“L”) - Arboreal vegetation of high stature that is less dense than other units; contains deciduous species that experience anthropic disturbance (a); stratum SSF2 (“M”) - Dense arboreal vegetation of high stature; well preserved forest with low quantity of deciduous species (b)

- the length of every element was measured with a tape along its central axis regardless of its shape (Figure 3.14);
- length measurements were only taken on the part of any individual element that exhibited a diameter large enough for inclusion into the CWD class;
- if the transect line crossed the branches of a forked tree, it counted as only one element; each branch’s diameter and length was measured individually for later volume calculation;
- the width of each element (perpendicular to the transect line; see Figure 3.16 and Figure 3.15) was measured;
- the elements’ decomposition stages were classified according to the HARMON *et al.* (1986) designations:

Class 1: Woody material consisting of solid wood, with leaves and/or fine twigs attached to the principal part, without noticeable degradation;

Class 2: Solid wood material with intact bark, but no leaves or fine twigs;

Class 3: Solid wood material similar to Class 2, except with rotting bark;

Class 4: Material somewhat rotten that can be broken when kicked;

Class 5: Material that is rotten, friable, and can be broken with little effort.

Figure 3.9: Mogi Guaçu Biological Reserve (CSS) map with grid points (300 x 300 m) (a) and selected points (b)

- when possible the plant species of an element was identified, though the identification process was uncertain for some of the elements. The bark of the element (attached or loose/hanging on the sides), branching pattern (if branches were still present), and/or the odor provided clues during this process (MARSHALL *et al.*, 2000).

3.2.2.4 Sample collection

Disc samples (i.e., cross-sectional cuts) were collected from all CWD elements at the transect line intersection point (Figure 3.17). For elements with a high degree of decomposition a manual saw was used (Figure 3.18a), and whenever necessary a tape was wrapped around the element to protect the sample from degradation during collection (Figure 3.18b). Samples were placed in a plastic bag, sealed with duct tape, and identified (Figure 3.19). All material collected in the field was transported to the “Luiz de Queiroz” College of Agriculture - ESALQ/USP, Department of Forest Sciences.

3.2.3 Laboratory analysis

3.2.3.1 Wood density determination

The wood density of the elements is required to convert volume per hectare (m^3/ha) to mass per hectare (kg/ha). Field measurements used to determine an element's wood density are based on CWD being intact, or in slabs, for sample collection, volume calculation, then a drying process to determine dry weight.

Figure 3.10: Mogi Guaçu Biological Reserve (CSS) map with inset views of strata and sampling units. Strata sampled include: forested savannah stratum in advanced stage (a/b) and forested savannah an intermediate stage (a/c)

Figure 3.11: The transect completely intersects a downed dead woody debris element. Source: pictures by Andréa B. Moreira

Figure 3.12: Intersection of a non-convex population element, U_k . In each transect, the open dot symbolizes the starting point. Examples of complete intersection (a); examples of partial intersection with the starting point of the line (b) and; examples of partial intersection by the front end of the line (c). Source: Adapted from GREGOIRE and VALENTINE (2008)

Figure 3.13: Diameter measurement of an element at the transect line intersection point; the pictures show two measurements - one parallel to the ground and the other perpendicular. Source: pictures by Andréa B. Moreira

Figure 3.14: Measuring the length of elements. Source: picture by Andréa B. Moreira

Figure 3.15: Perpendicular width measurement of a CWD element using a measuring tape. Source: picture by Andréa B. Moreira

The cylinders extraction method proposed by KELLER *et al.* (2004) was used to determine the density of each sample, with the only difference being that we conducted all extractions within a lab setting rather than at the field site. This process was performed at the Wood Technology Laboratory, Department of Forest Sciences, ESALQ/USP. For every disc sample collected, the extraction was performed using a cylindrical drill (removing a known volume of mass), attached to a drill bench (Figure 3.20). For elements

Figure 3.16: Perpendicular width (w_k) of the element U_k , the direction of the s^{th} transect line is represented by θ_s . Source: Adapted from GREGOIRE and VALENTINE (2008)

Figure 3.17: Disc samples extracted using chainsaw. Source: pictures by Andréa B. Moreira

with a high level of decay, cylinders were removed manually using an aluminum cylinder.

The cylinders (Figure 3.21) were extracted from the center (medulla) of the disc to the edge in one of the eight randomly selected positions along 5 cm intervals (KELLER *et al.*, 2004). The length of the cylinders was measured with a digital caliper as the length of the cylinders varied according to the width of the disc brought from the field. The cylinders of each sample were stored in containers marked with the sample code then dried in an oven at $65^\circ C$ until achieving a constant weight. The dry cylinders were removed from the oven and weighed on an analytical scale with a precision of four decimal places.

3.2.3.2 Carbon determination

Sample cylinders were milled in a knife mill (Wiley type), and sieved through a no. 40 mesh. Carbon content measurements were achieved using *LECO-C632* equipment at the Chemistry Integrated Laboratories, Pulp and Energy, Department of Forest Sciences, ESALQ/USP. During the process, the

Figure 3.18: Disc sample extracted with the use of a manual saw (a). Disc sample wrapped by the tape before cutting for extra protection (b). Source: picture by Andréa B. Moreira

Figure 3.19: Disc sample wrapped in plastic bag, sealed with duct tape, and identified. Source: pictures by Andréa B. Moreira

Figure 3.20: Cylinders extracted from the sample disc using a drill bench. Source: picture by Andréa B. Moreira

sample is combusted within a ceramic chamber in the presence of oxygen at temperatures around 1000°C for approximately one minute. After the combustion step, the sample exists in a combusted gaseous form that is filtered through an infrared sensor system to calculate the carbon content and convert it to a percentage value (by mass). For each sample, two replicates were conducted and averaged to determine carbon content.

Figure 3.21: Cylinders extracted from a sample disc with a drill bench; cylinders differ in color due to the varying levels of decomposition found in the same disc. Source: picture by Andréa B. Moreira

3.2.4 Data analysis

All data from both the field and the laboratories were analyzed using the statistical software R, version 3.3.2 (R CORE TEAM, 2016).

3.2.4.1 Volume

The cross-sectional area of each disc sample from each element (3.1) was calculated in square meters using the geometric mean of the two diameters measurements taken (dq_k).

$$g_k = \pi * \left(\frac{dq_k}{40000} \right)^2 \quad (3.1)$$

Hollow disc samples were photographed (Figure 3.22), and the hollowed areas were measured using the software *ImageJ* (RASBAND, 2016). The volume of each element was then calculated using following the equation (3.2):

$$V_k = (g_k - gh_k) \times l_k \quad (3.2)$$

where V_k is the volume of k^{th} sample element (m^3); g_k is the total area of the k^{th} sample element (m^2); gh_k is the sum of hollowed areas of the k^{th} sample (m^2); and l_k is the length of k^{th} sample element (m).

Figure 3.22: Samples discs with hollow. Source: picture by Andréa B. Moreira

3.2.4.2 Wood density

The wood density of each cylinder from all the samples collected were determined using the following equation:

$$\rho_{k_c} = \frac{dw_{k_c}}{V_{k_c}} \quad (3.3)$$

where: ρ_{k_c} is the wood density of the c^{th} cylinder of the k^{th} sample element (kg/m^3); dw_{k_c} is the dry weight of the c^{th} cylinder of the k^{th} sample element (kg); and V_{k_c} is the fresh volume of the c^{th} cylinder of the k^{th} sample element (m^3).

3.2.4.3 Biomass and carbon determination

The biomass (b_k) for each k^{th} element was determined by:

$$b_k = \rho_k \cdot V_k \quad (3.4)$$

The carbon content in each element was calculated as the product of its biomass and the average carbon content found in laboratory analysis by *LECO-C632* equipment.

3.2.4.4 Sampling estimators

Let the region $\mathcal{A}$ has horizontal area A , where there are N elements comprising the population. The orientation of the s^{th} line is indicated by θ_s , $s = 1, \dots, m_h$ ($m_h = 10$ for each h^{th} stratum). The sample selected by the s^{th} transect consisted of all population elements, U_k , intersected by the line of length L , following the protocol above.

The statistical properties of the estimators for the population parameters were determined using design-based inference where nothing is assumed about how y_k (a parameter of interest) values are distributed among N population units (GREGOIRE and VALENTINE, 2008). Each element of the population has its inclusion probability, π_k ; i.e., the probability of this element U_k being included in a possible sample $\mathbf{s}$. The inclusion probability of a resource unit, U_k , is directly related to its inclusion zone. The inclusion zone of each element has a constant width, $w_k(\theta_s)$, identical to the width of U_k in the perpendicular direction to the orientation of the line transect, θ_s . The area of the inclusion zone is $a_k = w_k(\theta_s) L$. Thus, the inclusion probability of U_k is:

$$\pi_k(\theta_s) = \frac{a_k}{A} = \frac{w_k(\theta_s) L}{A} \quad (3.5)$$

where perpendicular width, $w_k(\theta_s)$, and inclusion probability, $\pi_k(\theta_s)$, depend on the transect orientation; i.e., both measures are conditionally dependent on θ_s (KAISER, 1983; GREGOIRE and VALENTINE, 2008). For the cross shaped transect design, each segment (i.e., each line in the direction N-S or E-W), has half of the length of the entire transect, so the transect L length is the sum of the segments. In this case, the perpendicular width for the k^{th} element should be measured perpendicular to θ_s and $\theta_s^\perp$, even if the element is intersected by just one segment (GREGOIRE and VALENTINE, 2008). However, the inclusion probability would be:

$$\pi_k(\theta_s) = \frac{\bar{w}_k(\theta_s) L}{A} \quad (3.6)$$

where $\bar{w}_k(\theta_s) = (w_k(\theta_s) + w_k(\theta_s^\perp))/2$ (GREGOIRE and VALENTINE, 2008). In this study, only $w_k(\theta_s)$ was measured as it was not possible to calculate the average width.

HORVITZ and THOMPSON (1952) introduced an unbiased estimator for the total of a population parameter of interest. This general estimator, named the Horvitz-Thompson (HT) estimator, $\hat{\tau}_{y\pi}$,

expands each y_k measured in the sample by its inclusion probability. These expanded values are added together to serve as an estimator of τ_y , because $0 < \pi_k < 1$ (GREGOIRE and VALENTINE, 2008). In this study, four parameters of interest (y_k) were investigated: the number of elements, volume, biomass, and carbon. The estimated totals were calculated by area and by hectare for all possible sampling designs. In this way, the conditional HT estimator of the population total from the s^{th} transect (3.7) for the h^{th} stratum is:

$$\hat{\tau}_{y\pi sh}^c = \sum_{U_k \in sh} \frac{y_k}{\pi_k(\theta_s)} = \frac{A_h}{L} \sum_{U_k \in sh} \frac{y_k}{w_k(\theta_s)} \quad (3.7)$$

and therefore, the total per unit area (ha) would be calculated as:

$$\hat{\lambda}_{y\pi sh}^c = \frac{1}{A_h} \hat{\tau}_{y\pi sh}^c = \frac{1}{L} \sum_{U_k \in sh} \frac{y_k}{w_k(\theta_s)} \quad (3.8)$$

Since there are m_h independent estimates of $\hat{\tau}_{y\pi sh}^c$, $s = 1, \dots, m_h$, $m_h = 10$ for each stratum, we average them together to obtain an unbiased estimator of τ_{yh} . When we take a probability sample from the population at one location, and then replicate (“rep” subscript) that effort at $m - 1$ additional locations (GREGOIRE and VALENTINE, 2008), then:

$$\hat{\tau}_{y\pi h, rep}^c = \frac{1}{m_h} \sum_{s=1}^{m_h} \hat{\tau}_{y\pi sh}^c \quad (3.9)$$

and therefore:

$$\hat{\lambda}_{y\pi h, rep}^c = \frac{1}{m_h} \sum_{s=1}^{m_h} \hat{\lambda}_{y\pi sh}^c = \frac{\hat{\tau}_{y\pi h, rep}^c}{A_h} \quad (3.10)$$

The m_h estimates $\hat{\tau}_{y\pi sh}^c$, $s = 1, \dots, m_h$, provide direct, empirical evidence of how much the resource varies from one location to another in $\mathcal{A}$. Therefore, because replicated HT estimator is used, an unbiased estimator of the variance of $\hat{\tau}_{y\pi h, rep}^c$ for each h^{th} stratum can be calculated as (GREGOIRE and VALENTINE, 2008):

$$\hat{V}(\hat{\tau}_{y\pi h, rep}^c) = \frac{1}{m_h(m_h - 1)} \sum_{s=1}^{m_h} (\hat{\tau}_{y\pi sh}^c - \hat{\tau}_{y\pi h, rep}^c)^2 = \frac{s_{yh}^2}{m_h} \quad (3.11)$$

where $s_{yh}^2 = \frac{1}{(m_h - 1)} \sum_{s=1}^{m_h} (\hat{\tau}_{y\pi sh}^c - \hat{\tau}_{y\pi h, rep}^c)^2$ is the sample variance in the h^{th} stratum. Therefore:

$$\hat{V}(\hat{\lambda}_{y\pi h, rep}^c) = \frac{1}{m_h(m_h - 1)} \sum_{s=1}^{m_h} (\hat{\lambda}_{y\pi sh}^c - \hat{\lambda}_{y\pi h, rep}^c)^2 = \frac{1}{A_h^2} \hat{V}(\hat{\tau}_{y\pi h, rep}^c) \quad (3.12)$$

For each forest type, the Seasonal Semi-deciduous Forest and the Cerrado *sensu-stricto*, the population estimators for each variable (and their estimator of variance) for each stratum were calculated. The results were combined across the whole population. Since we have m_h estimates for each stratum, and two strata for each forest type, for the total population across the entire area we have $m = m_1 + m_2 = 10 + 10 = 20$ estimates (and we have a total area $A = A_1 + A_2$ hectares). Thus, the estimate of the population total of y is:

$$\hat{\tau}_{y\pi,rep}^c = \hat{\tau}_{y\pi1,rep}^c + \hat{\tau}_{y\pi2,rep}^c \quad (3.13)$$

therefore:

$$\hat{\lambda}_{y\pi,rep}^c = \frac{\hat{\tau}_{y\pi,rep}^c}{A} \quad (3.14)$$

And the respective estimators of the variance for $\hat{\tau}_{y\pi,rep}^c$ and $\hat{\lambda}_{y\pi,rep}^c$ are:

$$\hat{V}(\hat{\tau}_{y\pi,rep}^c) = \hat{V}(\hat{\tau}_{y\pi1,rep}^c) + \hat{V}(\hat{\tau}_{y\pi2,rep}^c) \quad (3.15)$$

therefore:

$$\hat{V}(\hat{\lambda}_{y\pi,rep}^c) = \frac{\hat{\tau}_{y\pi,rep}^c}{A^2} \quad (3.16)$$

The standard error of the estimator $\hat{\tau}_{y\pi,rep}^c$ is $\sqrt{\hat{V}(\hat{\tau}_{y\pi,rep}^c)}$, and both have the same units of measure. The estimated standard error of a parameter permits the construction of a confidence interval were computed for each sample, the fraction of intervals containing the target parameter τ_y would tend towards $(1 - \alpha)100\%$ (in this case, 90%):

$$\hat{\tau}_{y\pi,rep}^c \pm t_{1-\alpha/2, m-1} \sqrt{\hat{V}(\hat{\tau}_{y\pi,rep}^c)} \quad (3.17)$$

The estimated relative error was calculated by:

$$RE = \frac{\sqrt{\hat{V}(\hat{\tau}_{y\pi,rep}^c)}}{\hat{\tau}_{y\pi,rep}^c} 100\% \quad (3.18)$$

When estimating a per unit area basis, the estimated standard error of $\hat{\lambda}_{y\pi,rep}^c$ is $\sqrt{\hat{V}(\hat{\lambda}_{y\pi,rep}^c)}$, and the confidence interval is:

$$\hat{\lambda}_{y\pi,rep}^c \pm t_{1-\alpha/2, m-1} \sqrt{\hat{V}(\hat{\lambda}_{y\pi,rep}^c)}, \quad (3.19)$$

where the estimated relative error is:

$$RE = \frac{\sqrt{\hat{V}(\hat{\lambda}_{y\pi,rep}^c)}}{\hat{\lambda}_{y\pi,rep}^c} 100\% \quad (3.20)$$

Total estimators for the area and on a per hectare basis, their respective estimated standard deviations, confidence intervals, and estimated relative error were all calculated for each of the four y variables and for each sampling design. These estimations were used to compare and choose the best sampling design for each forest type — the smaller the estimated standard deviation, the narrower the distribution of the estimator, which means less variability for estimates from different samples. Therefore, the design with the narrowest interval estimate and the smallest estimated relative error will have the most accurate estimator. After selecting the best sampling design for each forest type, we estimated the four variables (number of elements, volume, biomass, and carbon stock) by decay class for the total area and on a per hectare basis.

3.3 Results and discussion

In the SSF type, 247 CWD elements were measured across the 10 sampling units in the SSF1 stratum, and 304 CWD elements across the 10 sampling units in the SSF2 stratum were measured. When possible, species identification was conducted; in total, 38 species belonging to 14 families were catalogued (see in the Appendix II). In the Mogi-Guaçu Biological Reserve (a CSS ecosystem), 132 CWD elements were sampled across 10 sampling units within the “forested savannah in an advanced successional stage” CSS1 stratum, and 77 CWD elements were sampled in the 10 sampling units within the CSS2 “forested savannah in an intermediate successional stage” stratum. For the elements where species could be identified, 18 species belonging to 15 families were catalogued — the most frequent was *Tapirira guianensis*, a typical Cerrado species (see in the Appendix I). In both forest types, elements from the genus *Ocotea* were identified — 110 times in the SSF but only four times in the CSS. Of note, it was not possible to discern the identification of *Ocotea* elements to the species level in the CSS type; only in the SSF type was species identification of elements from this genus possible (as in this forest type the species *Ocotea indecora* stood out among the others due to its characteristic odor; $n = 11$ elements with this characteristic). *Copaifera langsdorffii* and *Croton floribundus* were also identified in both forest types.

Table 3.1 shows the estimators combined from the two strata for the SSF type. For volume, biomass, and carbon stock estimation, the design with the smallest estimated standard error, narrowest confidence interval, and smallest relative error was the 200 m cross shaped transect. For the number of elements variable, the smallest relative error was the cross shaped transect with a line length of 150 m; however, the 200 m cross shaped transect design had the smallest estimated standard error and narrowest confidence interval. Same thing happened for number of elements of SSF2 stratum, for all the other variables and SSF1 200 m cross shaped was selected (Tables 3.2 and 3.3).

Table 3.4 presents the estimators calculated combined from the two strata for the CSS type. For all parameters of interest (frequency, volume, biomass, and carbon) the design with the smallest relative error, standard deviation, and confidence interval was the 150 m cross shaped transect. When analyzing the results by stratum (Tables 3.5 and 3.4) CSS1 presented the same result (150 m cross-shaped) for all four variables, but in the CSS2 for number of elements the selected design was 200 m cross-shaped, for volume 100 m cross-shaped had the smallest error, standard deviation and narrowest interval, and for biomass and carbon 100 m and 150 m cross-shaped showed close results.

GALVÃO FILHO (2010) did a similar study in the Amazon Forest testing three different transect shapes (square, cross, and L), with line lengths of 100, 150, and 200 m varied across groups of 5 or 9 sampling units. The study concluded that the best design was the L-shape with 150 m line lengths across five sampling units. DEUS (2015) also conducted sampling research in an Araucaria Forest where the LIS protocol was tested via 50 m transects and fixed area units of two sizes (50 x 50 m and 10 x 50 m); the 50 x 50 m fixed area plot showed the smallest sampling error.

Using the most accurate sampling design selected above for this research, the parameter estimates for the SSF type was 198 CWD elements/ha, 15.7 m³/ha of volume, 6.7 Mg/ha of biomass, and 3.3 Mg/ha of carbon. Our results match the values calculated for biomass found by KAUFFMAN *et al.* (1988) in Venezuela (5.6 Mg/ha) as well as those calculated by DEUS (2015) in an Araucaria Forest (5.3 Mg/ha). In a more recent study, CHAO *et al.* (2017) calculated 8.9 Mg/ha of biomass and 3.3 Mg C/ha for a tropical forest ecosystem in Taiwan, approximately is the same amount of carbon found in this study within the SSF. Observing the results by strata SSF1 has fewer number of CWD elements (183) than SSF2 (223), but for volume, biomass and carbon the values are close.

The parameter estimates for the CSS type was 95 CWD elements/ha, 3.3 m³/ha of volume, 1.3 Mg/ha of biomass, and 0.67 Mg/ha of carbon. LUCCA (2011) also estimated CWD element totals in a

Cerrado *sensu-stricto* area in São Paulo State, calculating 240 CWD elements/ha, 487 m³/ha of volume, and 0.2 Mg/ha of biomass. In the second year of that same research, parameter estimates were calculated as 141 CWD elements/ha, 293.5 m³/ha of volume, and 0.1 Mg/ha of biomass. The estimated number of CWD elements and volume values presented by LUGA (2011) are much greater than the ones found in this study. However, this trend does not follow for biomass, where our results were six times larger. Probably because CSS2 is in an intermediate successional stage, this area present smaller values in the four parameters when compared to CSS1 that it is in an advanced stage.

The biomass estimates found in this study are much smaller than the ones found in the Amazon Forest; e.g., 50.8 Mg/ha in KELLER *et al.* (2004); 58.5 Mg/ha in CHAO *et al.* (2009); 99.6 Mg/ha in RICE *et al.* (2004). The same circumstance exists for the carbon estimates: RICE *et al.* (2004) found 44.0 Mg C/ha, which is much higher than the estimates we computed. The small biomass estimates from our study fell in the range of other research in the tropics, such as that done in the Venezuelan Amazon (e.g., 1.6 - 48.5 Mg/ha; KAUFFMAN *et al.* (1988)) and also in Venezuela (0.65 - 42.3 Mg/ha; DELANEY *et al.* (1998)).

VEIGA (2010) and LUGA (2011) estimated CWD debris in the Atlantic Forest as well, both in the São Paulo State and specifically for the Ombrophilous Dense Forest forest type. LUGA (2011) investigated these area for two years and estimated 394 and 188 CWD elements/ha for the first two years, respectively (789.9 m³/ha and 190 m³/ha for the first two year's volume estimates, respectively), which are much higher than the values computed in this study; however, for biomass, LUGA (2011) calculated 0.67 and 0.23 Mg/ha of carbon, which are lower values compared to this study. VEIGA (2010), on the other hand, found 12.2 Mg/ha of biomass.

SANQUETTA *et al.* (2014) and SILVA (2013) also estimated dead woody biomass in Seasonal Semi-deciduous Forest areas. SANQUETTA *et al.* (2014) estimated biomass for CWD (diameter ≥ 7.51 cm) at 9 Mg/ha with a high proportion of carbon (42.1%) and 3.8 Mg C/ha; this final result is close to the estimates from our study, though the biomass estimate was likely higher probably because the samples included a greater number of CWD elements due to the different diameter classifications. SILVA (2013) computed slightly greater volume estimates (11.3 m³/ha) as well as biomass estimates (4.6 Mg/ha). Thus, overall, our results fall in the range of other studies on the Atlantic Forest.

Figure 3.23a shows the distribution of all 551 samples tallied from the 20 SSF sampling units. Clearly, the third decay class is the most frequent (56.8%), followed by the fourth class (26.3%). Figure 3.23b shows the distribution of all 209 elements measured from the 20 CSS sampling units. Again, the frequency of the third class stood out in relation to the other classes (56%). However, the difference between the densities in both forest types is apparent: the SSF type exhibited four times as many elements per hectare as the CSS type. Figures 3.24a and 3.24b show the distribution of the SSF type by stratum, SSF2 presented more elements by decay class than SSF1 probably because SSF2 is an area with more dense arboreal vegetation than SSF1. Figures 3.25a and 3.25b present the distribution of the elements from the CSS, where CSS1 that is the stratum in an advanced successional stage present more elements by decay class than CSS2 that is an area in intermediate successional stage.

Table 3.7 and Table 3.10 show the parameter estimates by hectare of CWD element frequency, biomass, volume, and carbon stock for each decay class in the SSF and CSS forest types, respectively. Tables 3.8 and 3.9 show the estimates for SSF1 and SSF2 strata by decay class, and Tables 3.11 and 3.12 for CSS1 and CSS2 strata by decay class. The CSS samples tallied only two elements in decay class 1; when sampling via the 150m cross shaped transect only one of these elements was collected and measured. Therefore, we were unable to calculate the estimators for this class. As expected, the third class exhibited the greatest quantities across all four variables, with the biomass being estimated at 5.0 Mg/ha in the SSF and 0.77 Mg/ha in the CSS. KELLER *et al.* (2004) showed this same behavior for volume and biomass in nine of the ten sites they studied. SANQUETTA *et al.* (2014) did not classify the

CWD elements into decay classes; SILVA (2013) used three classes, and VEIGA (2010) used four classes, but they both do not compute results by class.

The 200 m straight line transect design and the 100 m cross-shaped design both have the same total length of transect (200 m). In the SSF, the cross shaped transect had higher estimates and higher standard errors. The inverse happened in the CSS, though, as the straight line design had higher estimates and a higher standard error. These are an intriguing results observed as we had originally expected the values of both designs to be close, which only occurred for volume estimations in the CSS.

Table 3.1: Total sampling estimates for Seasonal Semi-deciduous Forest (SSF) type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (the two strata combined = 1217.3 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	307767.4	71937.8	183377.4	432157.3	252.8	59.1	150.6	355.0	23.4
150m S	297388.5	51950.9	207558.4	387218.5	244.3	42.7	170.5	318.1	17.5
200m S	283398.7	45527.5	204675.7	362121.8	232.8	37.4	168.1	297.5	16.1
100m C	302970.9	47529.7	220785.8	385156.1	248.9	39.0	181.4	316.4	15.7
150m C	270828.7	32271.7	215026.7	326630.8	222.5	26.5	176.6	268.3	11.9
200m C	240493.0	29814.5	188939.8	292046.1	197.6	24.5	155.2	239.9	12.4
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	24602.3	4614.1	16623.9	32580.7	20.2	3.8	13.7	26.8	18.8
150m S	23943.4	3422.7	18025.1	29861.8	19.7	2.8	14.8	24.5	14.3
200m S	22064.1	2841.5	17150.8	26977.4	18.1	2.3	14.1	22.2	12.9
100m C	28146.0	5372.5	18856.2	37435.8	23.1	4.4	15.5	30.8	19.1
150m C	23781.3	3181.1	18280.8	29281.9	19.5	2.6	15.0	24.1	13.4
200m C	19162.7	2043.0	15630.0	22695.3	15.7	1.7	12.8	18.6	10.7
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	11022.3	2277.2	7084.6	14959.9	9.1	1.9	5.8	12.3	20.7
150m S	10348.9	1613.6	7558.8	13139.0	8.5	1.3	6.2	10.8	15.6
200m S	9569.9	1290.1	7339.2	11800.7	7.9	1.1	6.0	9.7	13.5
100m C	12544.7	2727.6	7828.3	17261.0	10.3	2.2	6.4	14.2	21.7
150m C	10343.1	1399.6	7923.0	12763.1	8.5	1.1	6.5	10.5	13.5
200m C	8117.5	1007.6	6375.2	9859.8	6.7	0.8	5.2	8.1	12.4
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	5548.9	1203.2	3468.4	7629.3	4.6	1.0	2.8	6.3	21.7
150m S	5219.0	844.8	3758.2	6679.8	4.3	0.7	3.1	5.5	16.2
200m S	4807.1	669.8	3649.0	5965.3	3.9	0.6	3.0	4.9	13.9
100m C	6304.3	1364.4	3945.1	8663.5	5.2	1.1	3.2	7.1	21.6
150m C	5173.9	718.7	3931.2	6416.7	4.3	0.6	3.2	5.3	13.9
200m C	4067.5	514.5	3177.9	4957.1	3.3	0.4	2.6	4.1	12.6

Table 3.2: Total sampling estimates for the stratum SSF1 of the Seasonal Semi-deciduous Forest (SSF) type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (SSF1 = 777.9 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	199190.4	62618.3	84404.1	313976.8	256.1	80.5	108.5	403.6	31.4
150m S	187361.3	46110.6	102835.4	271887.1	240.9	59.3	132.2	349.5	24.6
200m S	157472.5	36142.2	91219.9	223725.1	202.4	46.5	117.3	287.6	23.0
100m C	186153.0	40175.7	112506.4	259799.5	239.3	51.6	144.6	334.0	21.6
150m C	151677.3	24005.8	107672.0	195682.6	195.0	30.9	138.4	251.6	15.8
200m C	142648.7	21446.8	103334.3	181963.0	183.4	27.6	132.8	233.9	15.0
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	14104.8	4086.0	6614.7	21594.8	18.1	5.3	8.5	27.8	29.0
150m S	14682.2	2910.7	9346.6	20017.8	18.9	3.7	12.0	25.7	19.8
200m S	13615.8	2342.1	9322.6	17909.1	17.5	3.0	12.0	23.0	17.2
100m C	11740.3	1984.3	8102.9	15377.7	15.1	2.6	10.4	19.8	16.9
150m C	13321.8	2343.2	9026.4	17617.2	17.1	3.0	11.6	22.6	17.6
200m C	11999.7	1619.3	9031.3	14968.1	15.4	2.1	11.6	19.2	13.5
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	6139.1	2023.4	2430.0	9848.3	7.9	2.6	3.1	12.7	33.0
150m S	6122.9	1396.9	3562.2	8683.6	7.9	1.8	4.6	11.2	22.8
200m S	5940.4	1087.8	3946.3	7934.6	7.6	1.4	5.1	10.2	18.3
100m C	4698.0	1029.0	2811.7	6584.2	6.0	1.3	3.6	8.5	21.9
150m C	5499.5	1022.2	3625.6	7373.4	7.1	1.3	4.7	9.5	18.6
200m C	5130.3	865.9	3543.0	6717.6	6.6	1.1	4.6	8.7	16.9
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	3122.7	1078.8	1145.1	5100.3	4.01	1.39	1.47	6.56	34.5
150m S	3097.8	733.9	1752.5	4443.1	3.98	0.94	2.25	5.71	23.7
200m S	2987.5	565.6	1950.6	4024.3	3.84	0.73	2.51	5.17	18.9
100m C	2374.9	543.5	1378.6	3371.2	3.05	0.70	1.77	4.33	22.9
150m C	2730.4	523.1	1771.5	3689.3	3.51	0.67	2.28	4.74	19.2
200m C	2581.5	442.7	1770.0	3393.1	3.32	0.57	2.28	4.36	17.1

Table 3.3: Total sampling estimates for the stratum SSF2 of the Seasonal Semi-deciduous Forest (SSF) type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (SSF2 = 439.4 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	108576.9	35411.8	43663.1	173490.7	247.1	80.6	99.4	394.8	32.6
150m S	110027.2	23931.5	66158.2	153896.3	250.4	54.5	150.6	350.2	21.8
200m S	125926.2	27685.7	75175.2	176677.2	286.6	63.0	171.1	402.1	22.0
100m C	116818.0	25396.6	70263.2	163372.8	265.9	57.8	159.9	371.8	21.7
150m C	119151.4	21568.2	79614.5	158688.3	271.2	49.1	181.2	361.2	18.1
200m C	97844.3	20710.8	59879.0	135809.6	222.7	47.1	136.3	309.1	21.2
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	10497.5	2143.5	6568.2	14426.9	23.9	4.9	14.9	32.8	20.4
150m S	9261.2	1800.8	5960.1	12562.4	21.1	4.1	13.6	28.6	19.4
200m S	8448.3	1609.0	5498.7	11397.8	19.2	3.7	12.5	25.9	19.0
100m C	16405.6	4992.7	7253.5	25557.8	37.3	11.4	16.5	58.2	30.4
150m C	10459.5	2151.4	6515.7	14403.4	23.8	4.9	14.8	32.8	20.6
200m C	7163.0	1245.7	4879.5	9446.4	16.3	2.8	11.1	21.5	17.4
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	4883.2	1044.8	2967.9	6798.4	11.1	2.4	6.8	15.5	21.4
150m S	4226.0	807.6	2745.5	5706.5	9.6	1.8	6.2	13.0	19.1
200m S	3629.5	693.5	2358.3	4900.8	8.3	1.6	5.4	11.2	19.1
100m C	7846.7	2526.0	3216.2	12477.2	17.9	5.7	7.3	28.4	32.2
150m C	4843.6	955.9	3091.3	6596.0	11.0	2.2	7.0	15.0	19.7
200m C	2987.2	515.3	2042.6	3931.7	6.8	1.2	4.6	8.9	17.2
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	2426.2	532.7	1449.7	3402.7	5.52	1.21	3.30	7.74	22.0
150m S	2121.2	418.5	1354.0	2888.4	4.83	0.95	3.08	6.57	19.7
200m S	1819.7	358.8	1162.0	2477.3	4.14	0.82	2.64	5.64	19.7
100m C	3929.5	1251.5	1635.4	6223.5	8.94	2.85	3.72	14.16	31.8
150m C	2443.5	492.8	1540.1	3347.0	5.56	1.12	3.51	7.62	20.2
200m C	1486.0	262.1	1005.5	1966.4	3.38	0.60	2.29	4.48	17.6

Table 3.4: Total sampling estimates for Cerrado *sensu-stricto* (CSS) forest type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (the two strata combined = 334 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	40277.7	11258.2	20810.7	59744.6	120.6	33.7	62.3	178.9	28.0
150m S	42402.2	8527.6	27656.8	57147.6	127.0	25.5	82.8	171.1	20.1
200m S	40418.5	8096.1	26419.3	54417.8	121.0	24.2	79.1	162.9	20.0
100m C	32950.8	6329.3	22006.6	43895.0	98.7	19.0	65.9	131.4	19.2
150m C	31763.9	4461.6	24049.1	39478.7	95.1	13.4	72.0	118.2	14.0
200m C	32933.2	4796.7	24639.0	41227.3	98.6	14.4	73.8	123.4	14.6
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	1157.7	350.7	551.4	1764.0	3.5	1.0	1.7	5.3	30.3
150m S	1297.1	409.0	589.8	2004.4	3.9	1.2	1.8	6.0	31.5
200m S	1505.8	416.6	785.4	2226.2	4.5	1.2	2.4	6.7	27.7
100m C	1411.7	391.7	734.5	2089.0	4.2	1.2	2.2	6.3	27.7
150m C	1094.4	216.2	720.6	1468.2	3.3	0.6	2.2	4.4	19.8
200m C	1393.2	289.0	893.6	1892.8	4.2	0.9	2.7	5.7	20.7
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	468.6	146.1	216.0	721.1	1.4	0.4	0.6	2.2	31.2
150m S	501.6	139.3	260.7	742.6	1.5	0.4	0.8	2.2	27.8
200m S	597.6	168.3	306.6	888.6	1.8	0.5	0.9	2.7	28.2
100m C	525.1	128.6	302.8	747.4	1.6	0.4	0.9	2.2	24.5
150m C	446.5	82.9	303.2	589.8	1.3	0.2	0.9	1.8	18.6
200m C	588.4	128.4	366.4	810.4	1.8	0.4	1.1	2.4	21.8
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	236.9	75.0	107.2	366.7	0.71	0.22	0.32	1.10	31.7
150m S	252.9	70.7	130.6	375.2	0.76	0.21	0.39	1.12	28.0
200m S	296.3	84.2	150.7	441.9	0.89	0.25	0.45	1.32	28.4
100m C	261.8	64.1	151.0	372.5	0.78	0.19	0.45	1.12	24.5
150m C	224.4	41.7	152.3	296.5	0.67	0.12	0.46	0.89	18.6
200m C	293.2	63.9	182.7	403.6	0.88	0.19	0.55	1.21	21.8

Table 3.5: Total sampling estimates for the stratum CSS1 of the Cerrado *sensu-stricto* (CSS) forest type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (CSS1 = 136.5 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	21148.9	4144.0	13552.5	28745.3	154.9	30.4	99.3	210.6	19.6
150m S	19918.4	4220.8	12181.1	27655.6	145.9	30.9	89.2	202.6	21.2
200m S	20868.6	5960.0	9943.1	31794.1	152.9	43.7	72.8	232.9	28.6
100m C	16789.4	2499.5	12207.5	21371.4	123.0	18.3	89.4	156.6	14.9
150m C	16546.6	2460.4	12036.5	21056.7	121.2	18.0	88.2	154.3	14.9
200m C	16600.4	3618.8	9966.6	23234.1	121.6	26.5	73.0	170.2	21.8
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	473.1	139.8	216.8	729.3	3.5	1.0	1.6	5.3	29.5
150m S	520.4	147.9	249.3	791.6	3.8	1.1	1.8	5.8	28.4
200m S	691.1	266.4	202.9	1179.4	5.1	2.0	1.5	8.6	38.5
100m C	868.7	365.1	199.5	1537.8	6.4	2.7	1.5	11.3	42.0
150m C	540.3	117.0	325.8	754.8	4.0	0.9	2.4	5.5	21.7
200m C	719.2	191.9	367.4	1070.9	5.3	1.4	2.7	7.8	26.7
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	209.9	80.8	61.7	358.0	1.5	0.6	0.5	2.6	38.5
150m S	217.6	70.4	88.6	346.5	1.6	0.5	0.6	2.5	32.3
200m S	291.6	121.8	68.3	515.0	2.1	0.9	0.5	3.8	41.8
100m C	327.1	113.9	118.2	535.9	2.4	0.8	0.9	3.9	34.8
150m C	221.3	49.2	131.1	311.6	1.6	0.4	1.0	2.3	22.2
200m C	323.4	88.0	162.2	484.7	2.4	0.6	1.2	3.6	27.2
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	106.9	42.9	28.3	185.6	0.78	0.31	0.21	1.36	40.1
150m S	110.0	36.6	42.9	177.1	0.81	0.27	0.31	1.30	33.3
200m S	146.5	61.8	33.2	259.8	1.07	0.45	0.24	1.90	42.2
100m C	163.0	56.4	59.7	266.3	1.19	0.41	0.44	1.95	34.6
150m C	111.0	24.8	65.5	156.5	0.81	0.18	0.48	1.15	22.4
200m C	162.5	44.5	80.9	244.1	1.19	0.33	0.59	1.79	27.4

Table 3.6: Total sampling estimates for the stratum CSS2 of the Cerrado *sensu-stricto* (CSS) forest type of the four parameter of interest (number of CWD elements, volume, biomass, and carbon), with values by total area (CSS2 = 197.5 ha) and by hectare, standard error (SE), 90% confidence intervals (CI), and relative error ($\hat{RE}$) as a percentage. Sampling designs have three possible transect lengths (100 m, 150 m, and 200 m) and two possible designs - a straight line design (S), and a cross shape design (C)

NUMBER OF ELEMENTS									
Design\ Units	Total/area (n)	SE (n)	CI		Total/ha (n/ha)	SE (n/ha)	CI		$\hat{RE}$ (%)
			low (n)	high (n)			low (n/ha)	high (n/ha)	
100m S	19128.8	10467.8	-59.9	38317.5	96.9	53.0	-0.3	194.0	54.7
150m S	22483.9	7409.8	8900.9	36066.8	113.8	37.5	45.1	182.6	33.0
200m S	19549.9	5479.5	9505.4	29594.4	98.0	27.7	48.1	149.9	28.0
100m C	16161.4	5814.8	5502.1	26820.6	81.8	29.4	27.9	135.8	36.0
150m C	15217.3	3722.0	8394.6	22040.1	77.1	18.8	42.5	111.6	24.5
200m C	16332.8	3148.4	10561.4	22104.2	82.7	15.9	53.5	111.9	19.3
VOLUME									
Design\ Units	Total/area (m ³)	SE (m ³)	CI		Total/ha (m ³ /ha)	SE (m ³ /ha)	CI		$\hat{RE}$ (%)
			low (m ³)	high (m ³)			low (m ³ /ha)	high (m ³ /ha)	
100m S	684.6	321.6	95.1	1274.1	3.5	1.6	0.5	6.5	47.0
150m S	776.7	381.4	77.6	1475.7	3.9	1.9	0.4	7.5	49.1
200m S	814.7	320.3	227.5	1401.9	4.1	1.6	1.2	7.1	39.3
100m C	543.1	142.0	282.8	803.3	2.7	0.7	1.4	4.1	26.1
150m C	554.0	181.8	220.8	887.2	2.8	0.9	1.1	4.5	32.8
200m C	674.1	216.0	278.1	1070.1	3.4	1.1	1.4	5.4	32.0
BIOMASS									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	258.7	121.7	35.7	481.8	1.3	0.6	0.2	2.4	47.0
150m S	284.1	120.3	63.7	504.5	1.4	0.6	0.3	2.6	42.3
200m S	306.0	116.1	93.1	518.8	1.5	0.6	0.5	2.6	38.0
100m C	198.0	59.6	88.8	307.3	1.0	0.3	0.4	1.6	30.1
150m C	225.2	66.7	103.0	347.4	1.1	0.3	0.5	1.8	29.6
200m C	265.0	93.5	93.5	436.4	1.3	0.5	0.5	2.2	35.3
CARBON									
Design\ Units	Total/area (Mg)	SE (Mg)	CI		Total/ha (Mg/ha)	SE (Mg/ha)	CI		$\hat{RE}$ (%)
			low (Mg)	high (Mg)			low (Mg/ha)	high (Mg/ha)	
100m S	130.0	61.5	17.2	242.8	0.66	0.31	0.09	1.23	47.3
150m S	142.9	60.5	32.0	253.9	0.72	0.31	0.16	1.29	42.3
200m S	149.8	57.2	44.9	254.6	0.76	0.29	0.23	1.29	38.2
100m C	98.8	30.5	42.9	154.6	0.50	0.15	0.22	0.78	30.8
150m C	113.4	33.5	52.0	174.8	0.57	0.17	0.26	0.89	29.5
200m C	130.7	45.8	46.7	214.7	0.66	0.23	0.24	1.09	35.1

Figure 3.23: Distribution of number of elements by decay class of all samples measured in the sampling units within the Seasonal Semi-deciduous Forest (a) and in the Cerrado *sensu-stricto* (b)

Figure 3.24: Distribution of number of elements by decay class of all samples measured in the sampling units within the Seasonal Semi-deciduous Forest stratum I (SSF1) (a) and stratum II (SSF2) (b)

Figure 3.25: Distribution of number of elements by decay class of all samples measured in the sampling units within the Cerrado *sensu-stricto* advanced stratum (CSS1) (a) and intermediate stratum (CSS2) (b)

Table 3.7: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the Seasonal Semi-deciduous Forest (SSF) type, the standard error in parenthesis, and the sample size (n) - using 200 m cross-shaped design

		Decay class				
		1	2	3	4	5
		($n=8$)	($n=13$)	($n=196$)	($n=80$)	($n=22$)
Number of elements	(n/ha)	1.54 (0.85)	6.33 (2.75)	96.72 (15.13)	67.02 (8.16)	25.96 (6.10)
Volume	(m ³ /ha)	0.32 (0.31)	0.59 (0.31)	11.05 (1.39)	3.05 (0.85)	0.72 (0.20)
Biomass	(Mg/ha)	0.19 (0.23)	0.30 (0.18)	5.02 (0.70)	1.01 (0.34)	0.14 (0.08)
Carbon	(Mg/ha)	0.10 (0.12)	0.16 (0.09)	2.50 (0.36)	0.52 (0.18)	0.07 (0.04)

Table 3.8: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the stratum SSF1 of Seasonal Semi-deciduous Forest (SSF) type, the standard error in parenthesis, and the sample size (n) - using 200 m cross-shaped design

		Decay class				
		1	2	3	4	5
		($n=2$)	($n=8$)	($n=89$)	($n=42$)	($n=12$)
Number of elements	(n/ha)	1.06 (1.28)	8.34 (4.09)	81.87 (13.88)	65.53 (10.75)	26.58 (7.68)
Volume	(m ³ /ha)	0.26 (0.44)	0.36 (0.11)	10.14 (1.74)	3.79 (1.32)	0.87 (0.31)
Biomass	(Mg/ha)	0.18 (0.34)	0.18 (0.06)	4.76 (0.93)	1.30 (0.52)	0.18 (0.12)
Carbon	(Mg/ha)	0.90 (0.17)	0.09 (0.03)	2.37 (0.48)	0.67 (0.27)	0.09 (0.06)

Table 3.9: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the stratum SSF2 of Seasonal Semi-deciduous Forest (SSF) type, the standard error in parenthesis, and the sample size (n) - using 200 m cross-shaped design

		Decay class				
		1	2	3	4	5
		($n=6$)	($n=13$)	($n=107$)	($n=38$)	($n=10$)
Number of elements	(n/ha)	2.39 (0.63)	2.79 (2.36)	123.0 (33.96)	69.64 (12.20)	24.88 (10.04)
Volume	(m ³ /ha)	0.44 (0.33)	1.01 (0.83)	12.66 (2.33)	1.75 (0.37)	0.46 (0.11)
Biomass	(Mg/ha)	0.21 (0.19)	0.53 (0.50)	5.49 (1.03)	0.49 (0.12)	0.08 (0.02)
Carbon	(Mg/ha)	0.11 (0.10)	0.27 (0.26)	2.72 (0.52)	0.25 (0.06)	0.04 (0.01)

Table 3.10: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the Cerrado *sensu-stricto* (CSS) forest type, the standard error in parenthesis, and the sample size (n) - using 150 m cross-shaped design

		Decay class			
		2	3	4	5
		($n=10$)	($n=44$)	($n=26$)	($n=11$)
Number of elements	(n/ha)	4.52 (1.97)	40.00 (10.57)	29.35 (5.02)	20.85 (10.12)
Volume	(m ³ /ha)	0.42 (0.32)	1.79 (0.47)	0.77 (0.38)	0.27 (0.09)
Biomass	(Mg/ha)	0.21 (0.17)	0.77 (0.16)	0.29 (0.17)	0.06 (0.02)
Carbon	(Mg/ha)	0.10 (0.08)	0.39 (0.08)	0.15 (0.08)	0.03 (0.01)

Table 3.11: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the stratum CSS1 of Cerrado *sensu-stricto* (CSS) forest type, the standard error in parenthesis, and the sample size (n) - using 150 m cross-shaped design

		Decay class			
		2	3	4	5
		($n=6$)	($n=31$)	($n=17$)	($n=4$)
Number of elements	(n/ha)	6.04 (3.26)	51.45 (10.06)	53.51 (9.79)	9.27 (0.75)
Volume	(m ³ /ha)	0.63 (0.59)	1.96 (0.48)	1.02 (0.41)	0.31 (0.14)
Biomass	(Mg/ha)	0.27 (0.25)	0.88 (0.22)	0.39 (0.19)	0.05 (0.00)
Carbon	(Mg/ha)	0.13 (0.12)	0.45 (0.12)	0.20 (0.09)	0.02 (0.00)

Table 3.12: Total estimates by hectare of the number of CWD elements, volume, biomass, and carbon content by decay class for the stratum CSS2 of Cerrado *sensu-stricto* (CSS) forest type, the standard error in parenthesis, and the sample size (n) - using 150 m cross-shaped design

		Decay class			
		2	3	4	5
		($n=4$)	($n=13$)	($n=9$)	($n=7$)
Number of elements	(n/ha)	3.47 (2.46)	32.09 (16.47)	12.64 (5.13)	28.85 (17.11)
Volume	(m ³ /ha)	0.28 (0.36)	1.68 (0.72)	0.60 (0.58)	0.25 (0.12)
Biomass	(Mg/ha)	0.16 (0.23)	0.69 (0.22)	0.22 (0.25)	0.06 (0.03)
Carbon	(Mg/ha)	0.08 (0.11)	0.35 (0.11)	0.11 (0.13)	0.03 (0.01)

3.4 Conclusion

Line intersect sampling is a very useful methodology for quantifying coarse woody debris, and it enables a design-unbiased estimation. None of the studies mentioned in the discussion of this paper used design-based inference or perpendicular width to estimate the parameters. Other sampling techniques used in forest inventory like plot sampling and horizontal point sampling (Bitterlich sampling) are applied through a design-based inference with the use of the Horvitz-Thompson estimator, and there is no necessity to be different with LIS method. Even with the use of perpendicular width – which means

one extra measure in the field – the classical sampling theory is recommended as KAISER (1983) affirms the population is fixed, i.e., as happen in practice the elements can have any spatial arrangement and any shape (not only needle shape), and the randomness enters the problem only through the sampling scheme.

For the SSF type, the most accurate design was the 200 m cross shaped transect. For the CSS type, the cross shaped transect design was also the best, but with the 150 m transect lengths instead. These designs are thus recommended for this type of study, but more studies need to be made to firmly conclude that they are the most optimal designs for measuring coarse woody debris in these types of forests. For more precise volume estimation, future inventories should measure the diameter at each CWD element's small-end and large-end. Also in the case of a cross or L-shaped transect design, both directions of perpendicular width need to be measured; i.e., the first measurement should be taken, then a 90° rotation should be performed, and then the second measurement should be taken at the new alignment.

Both forest types showed much less biomass (1.3 and 6.7 Mg/ha biomass, CSS and SSF, respectively), compared to other studies in the Amazon Forest where most of the CWD studies have been conducted. The SSF data produced estimates similar to the other two studies concentrating on the same type of vegetation (SILVA, 2013; SANQUETTA *et al.*, 2014). Only one other study of CWD has been conducted in a Cerrado ecosystem, and the results between this study and ours showed great differences. According to CHAO *et al.* (2009), greater values of biomass suggest greater dead wood accumulation. The authors also suggest, and so this study, that forest type can influence the quantity of CWD, and ignoring this information can lead to either over- or underestimation of necromass stocks.

This kind of study is very important for determining the appropriate sampling design protocol for future inventories. More studies are necessary in these two types of ecosystems to better understand how much CWD pools contribute to biomass and carbon stocks. The Cerrado *sensu-stricto* ecosystem in particular needs more attention, specifically across geographic variations of this ecosystem type. Also, future research should be guided by a similar field protocol to make more precise comparisons.

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Appendices

Appendix I -Family, scientific name, common name (in Portuguese), and number of CWD elements sampled (*n*) of the species identified in the Cerrado *sensu-stricto* forest type (Mogi Guaçu Biological Reserve)

Family	Specie	Commom name	<i>n</i>
Anacardiaceae	<i>Tapirira guianensis</i> Aubl.	peito de pomba	42
Annonaceae	<i>Annona crassiflora</i> Mart.	marolo	2
Annonaceae	<i>Xylopia aromatica</i> (Lam.) M.C. Dias	pimenta de macaco	2
Apocynaceae	<i>Aspidosperma tomentosum</i>	peroba do campo	1
Boraginaceae	<i>Cordia sellowiana</i> Cham.	baba de boi	1
Chrysobalanaceae	<i>Licania humilis</i> Cham. & Schltdl.	poleiro de corvo	1
Ebenaceae	<i>Diospyros hispida</i> A. DC	caqui do campo	1
Euphorbiaceae	<i>Croton floribundus</i> Spreng.	croton	2
Fabaceae	<i>Anadenanthera falcata</i> Speg.	angico	19
Fabaceae	<i>Copaifera langsdorffii</i> Desf.	óleo de copaíba	11
Lauraceae	<i>Ocotea</i> sp	canela	4
Malvaceae	<i>Pseudobombax longiflorum</i> (Mart. & Zucc.) A. Robyns	embiruçu	1
Melastomataceae	<i>Tibouchina stenocarpa</i> (Schrank & Mart. ex DC.) Cogn	quaresmeira	1
Meliaceae	<i>Guarea guidonia</i> (L.) Sleumer	guarea	1
Moraceae	<i>Ficus</i> sp	figueira	1
Sapotaceae	<i>Pouteria ramiflora</i> (Mart.) Radlk.	brasa viva	1
Vochysiaceae	<i>Qualea</i> sp.	qualea/pau terra	31
Vochysiaceae	<i>Vochysia tucanorum</i> Mart.	cinzeiro	13

Appendix II - Family, scientific name, common name (in Portuguese), and number of CWD elements sampled (*n*) of the species identified in the Seasonal Semi-deciduous Forest type (Caetetus Ecological Station)

Family	Specie	Commom name	<i>n</i>
Annonaceae	<i>Annona cacans</i> Warm.	araticum cagão	1
Annonaceae	<i>Duguetia lanceolata</i> A.St.-Hil.	pindaíba	1
Apocynaceae	<i>Aspidosperma polyneuron</i> Müll.Arg.	peroba	36
Araliaceae	<i>Schefflera morototonii</i> (Aubl.) Maguire, Steyerl. & Frodin	mandiocão	2
Asteraceae	<i>Piptocarpha sellowii</i> (Sch. Bip.) Baker	vassourão	5
Boraginaceae	<i>Cordia ecalyculata</i> Vell.	café de bugre	3
Boraginaceae	<i>Cordia superba</i> Cham.	babosa branca	1
Boraginaceae	<i>Cordia trichotoma</i> (Vell.) Arrab. ex Steud.	louro pardo	3
Combretaceae	<i>Terminalia glabrescens</i> Mart.	capitão	2
Euphorbiaceae	<i>Alchornea triplinervia</i> (Spreng.) M. Arg.	tapiá	2
Euphorbiaceae	<i>Aparisthium cordatum</i> (A.Juss.) Baill	tapiá	3
Euphorbiaceae	<i>Croton floribundus</i> Spreng.	capixingui	43
Euphorbiaceae	<i>Mabea fistulifera</i> Mart.	mamoninha do mato	5
Euphorbiaceae	<i>Margaritaria nobilis</i> L. f.	margaritaria	5
Euphorbiaceae	<i>Savia dictyocarpa</i> Müll.Arg.	guaraiuva	20
Fabaceae	<i>Calliandra foliolosa</i> Benth.	cabelo de anjo	1
Fabaceae	<i>Centrolobium tomentosum</i> Guill. ex Benth.	araribá	11
Fabaceae	<i>Copaifera langsdorffii</i> Desf.	óleo de copaíba	2
Fabaceae	<i>Enterolobium contortisiliquum</i> (Vell.) Morong	timburi	1
Fabaceae	<i>Inga striata</i> Benth.	ingá	16
Fabaceae	<i>Machaerium brasiliense</i> Vogel	sapuva	6
Fabaceae	<i>Myroxylon peruiferum</i> L. f.	cabreúva	1
Fabaceae	<i>Piptadenia gonoacantha</i> (Mart.) J.F.Macbr.	jacaré	37
Fabaceae	<i>Senegalia polyphylla</i> (DC.) Britton & Rose	monjoleiro	8
Lauraceae	<i>Ocotea</i> sp	canela	110
Lauraceae	<i>Ocotea indecora</i> (Shott) Mez	canela cheirosa	11
Lecythidaceae	<i>Cariniana estrellensis</i> (Raddi) Kuntze	jequitibá	2
Meliaceae	<i>Trichilia claussenii</i> C.DC.	catiguá	3
Myrtaceae	<i>Calyptranthes clusiifolia</i> O.Berg	araçarana	1
Myrtaceae	<i>Campomanesia xanthocarpa</i> O.Berg	guabiroba	1
Myrtaceae	<i>Myrcianthes pungens</i> (O.Berg) D. Legrand	eugênia florida	5
Rhamnaceae	<i>Colubrina grandulosa</i> Perkins	sobrasil	3
Rhamnaceae	<i>Rhamnidium elaeocarpum</i> Reissek	saguaraji amarelo	1
Rutaceae	<i>Balfourodendron riedelianum</i> (Engl.) Engl.	marfim	2
Rutaceae	<i>Esenbeckia leiocarpa</i> Engl.	guarantã	5
Rutaceae	<i>Metrodorea nigra</i> A.St.-Hil.	carrapateira	1
Rutaceae	<i>Pilocarpus pauciflorus</i> A. St.-Hil.	jaborandi	1
Rutaceae	<i>Zanthoxylum</i> sp	mamica de porca	1

4 WOOD DENSITY AND CARBON CONCENTRATION OF COARSE WOODY DEBRIS IN NATIVE FORESTS, BRAZIL

Abstract

With the objective of increasing knowledge on carbon stocks, and thus improving the accuracy of published estimates, the present study explored wood density and carbon concentration of coarse woody debris by decay class in a Seasonal Semi-deciduous Forest (SSF) area in the Atlantic Rain Forest and in a Cerrado *sensu-stricto* (CSS) area. For this research, only large elements (diameter *geq* 10 cm), known as coarse woody debris (CWD), were considered. Data were collected according to the line intersect sampling (LIS) method. Two strata were chosen in each area, and in each stratum ten sampling units were installed according to a systematic sampling protocol. Each unit included: one 200 m transect line in the North-South direction, one 200 m transect line in the East-West direction (crossing the center point of the N-S transect), a 150 m line crossing the N-S line in the East-West direction at 25 m south of the center point, and a 50 m East-West line crossing the N-S line at 50 m south of the center point (for a total of 650 m of transect length per sampling unit). For each element, the diameter, length, and perpendicular width were recorded at the transect intersection point; each element was also classified into a decay class, and when possible the species was identified. Furthermore, disc samples were also taken from each element, from which cylinder samples were extracted then oven dried to determine density. These cylinders were milled and analyzed using a LECO-C632 to determine carbon content (as a mass percentage). The volume of each element was calculated by taking the difference between the cross-sectional area and the hollowed area, then multiplying by the element's length. Biomass was calculated by multiplying an element's volume by its density, and carbon stocks were calculated by multiplying biomass values by carbon factor computed via lab analysis. Data from the 650m line intersect sampling units were used to calculate wood density and carbon concentration averages across the areas. Cross shaped transects (with 150 m segments for the CSS, and 200 m segments for the SSF) were used to calculate estimators of biomass and carbon stock. In both areas, as the decay class increased (from least to most rotten material), wood density decreased, which follows the same pattern as other literature. The present study concludes that carbon concentration barely changes within each decay class. Biomass was estimated at 6.7 and 1.3 Mg/ha for the SSF and the CSS, respectively. Carbon stock concentrations reported from laboratory analyses were 3.3 and 0.7 MgC/ha for the SSF and the CSS, respectively. Using a conversion constant of 50% similar results were obtained, though other studies using the conversion constants found that they had overestimated carbon stock values. Due to this result, we strongly recommend the evaluation of wood density and carbon concentration for CWD at the site-level using decay classes.

Keywords: Carbon concentration; Wood density; Coarse woody debris; Seasonal Semi-deciduous Forest; Cerrado

4.1 Introduction

Forests play an important role in the carbon cycle, contributing to a substantial portion of the world's carbon stocks. Collectively, the world's forests store more than 650 billion tons of carbon: 44% in biomass, 11% in dead wood and litter, and 45% in the soil (FAO, 2010). Dead woody debris thus constitutes an important component of the carbon sequestration occurring in forests, and its quantification in forest inventories becomes even more relevant due to forests' interactions with the greenhouse effect and climate change (SMITH, 2004). As such, the assessment of coarse woody debris comprises an essential component of efficient forest management. Moreover, the quantification of carbon stocks improves our

understanding of climate change and the role of forest carbon dynamics in various future scenarios, and a more accurate quantification of dead woody material only increases the accuracy of overarching carbon estimates (WOODALL and WILLIAMS, 2005).

Despite this importance, carbon stock data for dead wood and litter remain vague (FAO, 2010). The woody debris studies that do exist focus on volume and biomass inventories, eschewing calculations on actual carbon concentrations (RUSSELL *et al.*, 2015). Instead, carbon stocks are indirectly estimated through volume estimators and biomass-carbon conversion constants (WOODALL and MONLEON, 2008). Because vegetation sequesters atmospheric carbon in biomass, which is commonly expressed as a dry weight per unit area, changes in net primary productivity (NPP) values over time often indicate changes in forest structure (BROWN, 1997). Biomass values are usually converted to carbon values by multiplying by the constant 0.50 (i.e., computed as 50% of the biomass value). The IPCC (2006) specifically recommends the use of a 47% biomass-carbon conversion factor for tropical rainforests when there is a dearth of more specific data specific to this forest type.

Dead woody debris (DWD) carbon stock estimates could improve by better understanding how carbon concentration varies in woody detritus, and DWD biomass estimates can become more precise when estimating wood density by decay class, species, position with respect to the soil surface, and tissue type (HARMON *et al.*, 2013). Carbon stocks can only be estimated precisely and in large-scale if there are detailed forest-type specifications for carbon concentration values (IPCC, 2006).

Carbon concentration studies of CWD in tropical forests by CHAO *et al.* (2017) note four previous publications pertinent to this type of research (CLARK *et al.*, 2002; IWASHITA *et al.*, 2013; MERIEM *et al.*, 2016) and WILCKE *et al.* (2005). No studies were found for the Cerrado ecosystem of Brazil, however. With the objective of increasing knowledge on carbon stocks, and thus improving the accuracy of published estimates, the present study explored wood density and carbon concentration of coarse woody debris by decay class in a Seasonal Semi-deciduous Forest area in the Atlantic Rain Forest and in a Cerrado *sensu-stricto* (Brazilian savannah) area.

4.2 Material and methods

4.2.1 Study sites

This study was conducted at two different areas located in two separate ecoregions of the São Paulo State, Brazil. The first area was within the Brazilian Atlantic Forest in a Seasonal Semi-deciduous Forest (SSF), also known as tropical seasonal forest, located inside the borders of the Caetetus Ecological Station. The second area was within the Cerrado ecoregion in an area classified as Cerrado *sensu-stricto* (CSS) located on the Mogi Guaçu Biological Reserve. In each area two strata were chosen for stratified systematic sampling.

The forest at the Caetetus Ecological Station constitutes one of the most significant remaining areas of SSF in Brazil. The “semi-deciduous” denomination refers to the seasonal cold weather and the reduced of water availability in the soil, alongside other environmental factors. As such, most of the arborescent species lose their leaves in winter to reduce water consumption and decrease their rates of growth (TABANEZ *et al.*, 2005). According to the Management Plan, the Conservation Unit covers the cities of Galia and Alvinlândia with a total area of 2178 ha. Its elevation ranges from 520-680 m above sea level, and it is dominated by lower sloped areas (with grades less than 6%). It is located on the northern border area of the hydrographic basin of the Paranapanema river. The climate according to the KÖEPPEN (1948) classification is “Cwa”: mesothermal dry winter with temperatures below 18° C and above 22° C in summer; total precipitation in the driest month is only 30 mm, and total annual precipitation is between 1100 and 1700 mm (TABANEZ *et al.*, 2005). In this area, stratum I (SSF1) and stratum II (SSF2) were selected.

The SSF1 stratum was characterized by arboreal vegetation of large stature with sparse herbaceous and graminoid vegetation. SSF1 contained predominantly deciduous species and has experienced anthropogenic disturbance; it occupies the interfluves and the tops of plateaus with a total area of 777.8 ha. This area was identified and mapped in the Management Plan as area “L”. The SSF2 stratum was an area containing dense arboreal vegetation of large stature with a smaller number of deciduous species. Overall, SSF2 was a well-preserved forest occupying high escarpments and plateau edges across a total area of 439.4 ha. It was identified and mapped in the Management Plan as area “M” (TABANEZ *et al.*, 2005).

The Mogi-Guaçu Biological Reserve, the location of the cerrado field site, is part of the old Campininha Farm located in the Martin Prado Junior district of the city of Mogi Guaçu in the São Paulo State. With a total area of 470 hectares, the landscape is relatively flat (less than a 15% slope). In the area of the Campininha Farm, the elevation ranges from 566 to 724 meters above sea level. The climate is mesothermal with two well defined seasons: a dry winter (from the months of April to September) and a hot summer (from October to March). The annual average rainfall is 1335 mm, and the average temperature is 20.5° C (SÃO PAULO, 2015). The unit is divided into two main sections: one designated as area “A” and the other as area “B”. Area “A”, where this research was conducted, covers 343.42 ha and is dominated by cerrado ecosystems at different regeneration stages, with the largest area being a Cerrado *sensu-stricto* (savanna woodland) formation (spanning from pioneer to advanced successional stages); there are also small patches of herbaceous vegetation near the watercourse. Two strata were also selected in this area: CSS1 and CSS2. The CSS1 stratum is characterized as a forested savannah in an advanced successional stage with an area of 136.5 ha; and the CSS2 stratum is a forested savannah in an intermediate successional stage with an area of 197.5 ha (SÃO PAULO, 2015).

4.2.2 Field measurements and sample collection

In the field survey, dead woody debris was classified into size classes according to the KELLER *et al.* (2004) classification: small (branches or bamboo with diameter between 2 and 5 cm); medium (branches or bamboo with diameter between 5 and 10 cm); and large (woody material with diameter greater than 10 cm). In this research, only the large elements, known as coarse woody debris (CWD), were considered. Data were collected according to the line intersect sampling (LIS) method, in which the sample unit is a transect, and downed dead wood were tallied following the field protocol rules detailed by GREGOIRE and VALENTINE (2008). In each stratum of each area, ten sampling units were installed according to a systematic sampling protocol (Figure 4.1), with 650 m of transect lines per sampling unit: one 200 m transect line in the North-South direction, one 200 m transect line in the East-West direction (crossing the center point of the N-S transect), a 150 m line crossing the N-S line in the East-West direction at 25 m south of the center point, and a 50 m East-West line crossing the N-S line at 50 m south of the center point.

A caliper was used to measure each element’s diameter at the point where the line intersected the element. If bark remained attached to the element, the diameter was measured with it. The length of the element was recorded using a tape measure along the central axis of the element (regardless of its shape). Furthermore, each element’s width perpendicular to the transect line direction was measured according to GREGOIRE and VALENTINE (2008). Each element was also classified into one of five decomposition stages according to HARMON *et al.* (1986): “class 1” (woody material consisting of solid wood with leaves and/or fine twigs attached to the principal part without noticeable degradation); “class 2” (solid wood material with intact bark but no leaves or fine twigs); “class 3” (solid wood material similar to “class 2” except with rotting bark); “class 4” (partially rotten material that can be broken when kicked); and “class 5” (material that is rotten, friable, and can be broken with bare hands). Though species identification for

debris material is difficult, this data was collected for CWD elements whenever possible – most frequently when the presence of bark and attached branches exhibited particular characteristics of certain species (e.g., a specific odor). For each element, a disc sample was collected at the transect line intersection point. These samples were taken using either a chain saw or a manual saw (for elements that had a high degree of decomposition). Samples were packaged, tagged, and carefully transported to the laboratory.

Figure 4.1: Sampling unit with a transect in the North-South direction (continuous line, 200 m), and three transects in the East-West direction (dashed lines)

4.2.3 Laboratory analysis

For density calculations from disc samples, the cylinder extraction method proposed by KELLER *et al.* (2004) was used, with the only variation in this study being that the cylinders were removed in a laboratory setting. The extraction of the cylinders was performed using a bench-mounted drill with a hole saw that produced samples of known diameter. For elements with a high level of decay, sample cylinders were removed manually using an aluminum cylinder. The length of the cylinders was measured and confirmed with a digital caliper; although the hole saw (drill bit) had a known volume, the length of the sample cylinders it produced varied according to the width of the disc brought from the field. The sample cylinders were oven-dried at $65^{\circ}C$ to a constant weight. Dry cylinders were removed from the oven and weighed to four decimal places on an analytical scale to compute final dry weight.

The sample cylinders from each disc were milled in a knife mill (Wiley type), and sieved through a no. 40 mesh, with equipment being vacuum-cleaned between samples to avoid contamination. Carbon content measurements were achieved with LECO-C632 carbon combustion and analysis equipment; i.e., samples are placed in a ceramic chamber that reaches a temperature of approximately $1000^{\circ}C$ for about one minute, undergoing combustion in the presence of oxygen. The LECO system uses infrared sensors to detect elemental carbon in the outflow of gas from the combustion system, and results are delivered as percentage mass values. For each sample, two replicates were made to determine final carbon content. All laboratory analyses were performed in the Department of Forest Sciences at the University of São Paulo (ESALQ/USP).

4.2.4 Data analysis

The data from the 650 m line intersect sampling units were used to calculate wood density and carbon concentration. The cross-shaped transects, specifically, were used as this sampling design was considered the optimal design (compared to straight line transects) according to their calculated estimators; 150 m segments were used in the CSS (Figure 4.1, blue lines), and 200 m segments were used in the SSF (Figure 4.1, red lines). The wood density (ρ_k) of each k^{th} element was computed as the average of the sample cylinders' densities, calculated as the dry weight (g) divided by fresh volume (cm^3); average densities were also computed for each decay class. For the volume calculation, discs with hollow sections were photographed, and the hollow areas were measured using *ImageJ* (RASBAND, 2016). Thus, the volume for each element (v_k) was calculated as: the cross-sectional area of the k^{th} element minus the hollowed cross-sectional area of the k^{th} element then multiplied by the length of the k^{th} element.

The biomass (b_k) value for each k^{th} element was determined as the product of the element's volume and its density: $b_k = V_k \cdot \rho_k$. The carbon content in each k^{th} element was calculated as the product of its biomass and the carbon concentration average computed via the laboratory analysis by *LECO-C632* equipment then averaged according to decay class. From the SSF area, $n = 506$ elements were analyzed for density and carbon concentration; from the CSS area, $n = 182$ elements were analyzed.

The total estimation of biomass/carbon concentration per hectare by sampling unit s ($\hat{\lambda}_{y\pi s}$) was calculated using the estimator $\hat{\lambda}_{y\pi s}^c = \sum_{U_k \in s} \frac{y_k}{w_k(\theta_s) L}$, where L is the length of both segments that makes the cross shaped design, y_k is the parameter of interest (biomass or carbon concentration), and w_k is the perpendicular width to the transect direction, (θ_s). The estimation for each unit was replicated, and all estimates were combined for the whole population. See details about the estimators in Chapter 3, as well as GREGOIRE and VALENTINE (2008). All data were processed and analyzed using the statistical software R (R CORE TEAM, 2016).

4.3 Results and discussion

For both areas, as the decay class increases (from freshest to most rotten material), wood density decreases. Table 4.1 shows the density results by stratum, as well as combined for each of the two study areas. Table 4.2 compares density values with other tropical forest and Cerrado studies. Prior research follows the general pattern that wood density decreases as decay class increases (i.e., the wooden debris becomes more rotten). Only KELLER *et al.* (2004) shows an exception to this trend: “class 2” exhibited a larger value than “class 1”; however, the authors did not propose an explanation for this observation. The rotten class from the SSF exhibited lower densities compared to the CSS, which matched the results from LUCCA (2011) in an Ombrophilous Dense Forest (0.17 g/cm^3). The SSF value was also similar to studies computing wood density ranges from 0.20 - 0.22 g/cm^3 , such as CHAO *et al.* (2017, 2008); WILCKE *et al.* (2005), and CLARK *et al.* (2002). In terms of the present study, only “class 5” showed differences in wood density between the two areas: in the CSS ($0.26 \pm 0.17 \text{ g/cm}^3$) the “class 5” density value was larger than the SSF value ($0.17 \pm 0.12 \text{ g/cm}^3$). IWASHITA *et al.* (2013) was the only study that showed a very low value for “class 5” (0.07 g/cm^3) compared to the other studies.

The range of wood density values computed indicate the importance of measuring wood density to achieve better accuracy for CWD biomass estimates (CHAO *et al.*, 2017). The Appendix I shows wood density and carbon concentration values by species and by decay class (but only when the species in the determined class was sampled five or more times). For some species, the general trend that a higher decay class means lower wood density did not hold. For *Tapirira guianensis* in the CSS and *Ocotea* sp. in the SSF, this circumstance occurred; and, similar to the results of KELLER *et al.* (2004), “class 3” was an order of magnitude larger than “class 2”. For *Savia dictyocarpa* in the SSF, “class 4” showed larger

density values than “class 3”. Though species identification for CWD is very difficult, if measurements on woody debris for specific species are available this data can help better understand variance patterns CHAO *et al.* (2017).

As in the current study, most studies classify CWD into five decomposition stages based on physical properties. Table 4.2 shows that some studies divide decay status into three or four stages, making the comparison more difficult. Our study produced results similar to those of CHAO *et al.* (2008) who investigated CWD (≥ 10 cm) in Amazon-Peru. They first divided elements into five decay classes, but they did not have data for “class 1” (due to limited sample size; $n=1$), as happened with both strata in the CSS area of this study. However, CHAO *et al.* (2008) combined classes “1” with “2”, and “4” with “5”, to draw comparisons across three classes and rendering results similar to classes “1”, “3”, and “5” of the current study. When results from the CSS area in the current study are compared with LUCCA (2011), with results also from Cerrado *sensu-stricto* vegetation, the values are similar for classes “1” and “3”, and Lucca’s are slightly higher for “class 5”.

As has been noted, few studies have investigated carbon concentration. Table 4.3 shows the carbon concentration found in both strata of both areas of the present study, and Table 4.4 compares these results with other studies in tropical forests. No study was found to have investigated carbon concentration of dead woody debris in a Cerrado *sensu-stricto* ecosystem. In the few studies reported for tropical forests there are contradicting results. The present study’s results parallel IWASHITA *et al.* (2013); MERIEM *et al.* (2016), and WILCKE *et al.* (2005) where the carbon concentration barely changes within the decay classes. CLARK *et al.* (2002) showed a small decrease in carbon concentration with an increase in decay class, and CHAO *et al.* (2017) found in both areas that the carbon concentration decreases significantly with the decay class.

CHAO *et al.* (2017) affirms that the variance of wood density and carbon concentration increases with decay classes; i.e., the higher the class, the higher the variability. Their hypothesis is that a fraction of fixed carbon is unlikely to be maintained in tropical forests with high biodiversity because of the many decomposition trajectories involving woody debris substrate quality, the myriad of microorganism activities, and the climatic conditions. HARMON *et al.* (1986) concludes decomposition is summarized in two major processes: physical/biological fragmentation of the material on the one hand, and mineralization (such as soil leaching and respiration) on the other.

Biomass was estimated at 6.67 ± 0.83 Mg/ha ($n=319$) and 1.34 ± 0.25 Mg/ha ($n=92$) for the SSF and the CSS, respectively. Carbon stock concentrations reported from laboratory analyses were 3.34 ± 0.42 MgC/ha and 0.67 ± 0.12 MgC/ha for the SSF and the CSS, respectively. With a conversion constant of 50%, similar results were obtained: 3.33 ± 0.41 MgC/ha for the SSF and 0.67 ± 0.12 MgC/ha for the CSS. CHAO *et al.* (2017), using similar analyses, found that they had overestimated carbon stock values by 16.8-35.6% when using the constant of 50%. Due to this result, they strongly recommended the evaluation of carbon concentration for CWD at the site-level, especially for tropical forests.

Table 4.1: Average wood density (g/cm^3), by decay class, of coarse woody debris in the strata of the two study areas, the Seasonal Semi-deciduous Forest (SSF) and the Cerrado *sensu-stricto* (CSS). The table shows the average wood density values with the calculated standard deviations in parentheses (with n being the sample sizes)

Decay class	Wood density (g/cm^3)					
	n_1	SSF1	n_2	SSF2	n	SSF Overall
1	3	0.48 (0.22)	10	0.55 (0.13)	13	0.54 (0.15)
2	14	0.45 (0.13)	19	0.50 (0.12)	33	0.48 (0.12)
3	130	0.46 (0.15)	174	0.46 (0.17)	304	0.46 (0.16)
4	61	0.32 (0.16)	63	0.28 (0.13)	124	0.30 (0.15)
5	17	0.19 (0.15)	15	0.15 (0.07)	32	0.17 (0.12)
		CSS1		CSS2		CSS Overall
1	1	0.69 (0.00)	1	0.49 (0.00)	2	0.59 (0.14)
2	12	0.44 (0.12)	6	0.46 (0.10)	18	0.45 (0.11)
3	71	0.46 (0.16)	38	0.45 (0.19)	109	0.46 (0.17)
4	28	0.32 (0.16)	18	0.33 (0.17)	46	0.32 (0.17)
5	4	0.25 (0.24)	3	0.26 (0.05)	7	0.26 (0.17)

Table 4.2: Wood density (g/cm^3) of woody debris found in the forestry literature for Tropical Forests and Cerrado ecosystems

Reference	Forest type	Country	Density (g/cm^3)					Criteria	Sample size
			Decay class 1	Decay class 2	Decay class 3	Decay class 4	Decay class 5		
This study	Seasonal semi-deciduous forest	Brazil	0.54	0.48	0.46	0.30	0.17	$\geq 10 \text{ cm}$	508
CHAO <i>et al.</i> (2017)	Tropical lowland windswept forest	Taiwan	0.41	0.36	0.33	0.31	0.24	$\geq 1 \text{ cm}$	378
CHAO <i>et al.</i> (2017)	Tropical lowland rainforest	Taiwan	0.37	0.32	0.27	0.22	0.20	$\geq 1 \text{ cm}$	357
IWASHITA <i>et al.</i> (2013)	Tropical montane wet forest	Hawaii	0.69	0.39		0.16	0.07	$\geq 2 \text{ cm}$	63
LUCCA (2011)	Ombrophilous dense forest	Brazil	0.43		0.28		0.17	$\geq 10 \text{ cm}$	-
VEIGA (2010)	Ombrophilous dense forest	Brazil	0.40	0.30	0.22	0.19	0.14	$\geq 10 \text{ cm}$	-
CHAO <i>et al.</i> (2008)	Amazon forest	Peru	-	0.53	0.41	0.28	0.21	$\geq 10 \text{ cm}$	251
CHAO <i>et al.</i> (2008)	Amazon forest	Peru	0.55		0.41		0.23	$\geq 10 \text{ cm}$	251
WILCKE <i>et al.</i> (2005)	Tropical lower montane forest	Ecuador	0.38				0.22	$\geq 10 \text{ cm}$	94
KELLER <i>et al.</i> (2004)	Amazon forest	Brazil	0.60	0.70	0.58	0.45	0.28	$\geq 10 \text{ cm}$	283
CLARK <i>et al.</i> (2002)	Tropical wet forest	Costa Rica	0.50		0.24		0.20	$\geq 10 \text{ cm}$	21
This study	Cerrado <i>sensu-stricto</i>	Brazil	0.59	0.45	0.46	0.32	0.26	$\geq 10 \text{ cm}$	185
LUCCA (2011)	Cerrado <i>sensu-stricto</i>	Brazil	0.62		0.44		0.36	$\geq 10 \text{ cm}$	-

Table 4.3: Average carbon concentration (%), by decay class, of coarse woody debris in the strata of the two study areas, the Seasonal Semideciduous Forest (SSF) and the Cerrado *sensu-stricto* (CSS). The table shows carbon concentration averages and the calculated standard deviations in parentheses (with n being the sample sizes)

Decay class	Carbon concentration (%)					
	n_1	SSF1	n_2	SSF2	n	SSF Overall
1	3	50.7 (0.36)	10	50.4 (1.46)	13	50.5 (1.27)
2	14	51.2 (1.46)	19	51.4 (1.42)	33	51.3 (1.42)
3	130	49.3 (3.20)	174	49.8 (2.92)	304	49.6 (3.05)
4	61	50.4 (3.64)	63	49.6 (4.42)	124	50.0 (4.06)
5	17	48.8 (4.02)	15	49.7 (3.04)	32	49.3 (3.56)
		CSS1		CSS2		CSS Overall
1	1	50.0 (0.00)	1	49.5 (0.00)	2	49.8 (0.35)
2	12	49.8 (0.81)	6	49.5 (0.80)	18	49.7 (0.80)
3	71	50.1 (1.46)	38	49.4 (3.13)	109	49.8 (2.19)
4	28	49.0 (4.40)	18	50.4 (2.01)	46	49.5 (3.68)
5	4	46.1 (2.32)	3	48.4 (0.35)	7	47.1 (2.06)

Table 4.4: Carbon concentration (%) of woody debris found in the forestry literature for Tropical Forests and Cerrado ecosystems

Reference	Forest type	Country	Carbon concentration (%)					Criteria	Sample size
			Decay class 1	Decay class 2	Decay class 3	Decay class 4	Decay class 5		
This study	Seasonal semi-deciduous forest	Brazil	50.5	51.3	49.6	50.0	49.2	≥10 cm	508
CHAO <i>et al.</i> (2017)	Tropical lowland windswept forest	Taiwan	37.6	41.1	36.6	28.7	15.8	≥ 1 cm	95
CHAO <i>et al.</i> (2017)	Tropical lowland rainforest	Taiwan	45.0	41.7	38.8	32.2	23.7	≥ 1 cm	95
MERIEU <i>et al.</i> (2016)	Tropical lowland rainforest	Indonesia	43.0		41.5		40.0	≥10 cm	261
IWASHITA <i>et al.</i> (2013)	Tropical montane wet forest	Hawaii	46.3	46.8		47.6	47.9	≥ 2 cm	48
WILCKE <i>et al.</i> (2005)	Tropical lower montane forest	Ecuador	46.8				47.2	≥10 cm	16
CLARK <i>et al.</i> (2002)	Tropical wet forest	Costa Rica	48.3		47.2		46.4	≥10 cm	21
This study	Cerrado <i>sensu-stricto</i>	Brazil	49.8	49.7	49.8	49.5	47.1	≥10 cm	185

4.4 Conclusion

Wood density and carbon concentration of dead woody debris are important variables for accurate estimation of biomass and carbon stocks. The present work supports numerous studies indicating that, in general, as wooden debris becomes more rotten (i.e., as its decay class increases) its density decreases. However, more extensive wood density and biomass details are needed for the Atlantic Rain Forest and the Cerrado (and, specifically, information on species).

Very few studies were found that measured the carbon concentration of dead woody debris, and most other studies use the conventional conversion factor of 50%. The present study did not find significant differences when calculating carbon concentrations using *in situ* data versus the 50% conversion method; but, there are contradictory results across the scientific literature, hence the recommendation of CHAO *et al.* (2017) to evaluate published data in order to establish carbon behavior patterns within decay classes for different forest types.

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Appendices

Appendix I - Wood density (g/cm³) and carbon concentration (%) averages of coarse woody debris by species and decay class for the Cerrado *sensu-stricto* (CSS) and Seasonal Semi-deciduous Forest (SSF) areas, with sample size $n \geq 5$, standard deviation in the parenthesis

Area	Specie	Decay class	n	Density (g/cm ³)	Carbon concentration (%)
CSS	<i>Anadenanthera falcata</i> Speg.	3	11	0.60 (0.14)	52.4 (1.36)
	<i>Copaifera langsdorffii</i> Desf.	3	5	0.48 (0.08)	49.5 (0.68)
	<i>Qualea</i> sp.	3	24	0.47 (0.21)	49.0 (3.75)
	<i>Tapirira guianensis</i> Aubl.	2	8	0.39 (0.05)	49.2 (0.30)
	<i>Tapirira guianensis</i> Aubl.	3	20	0.40 (0.15)	49.0 (1.15)
	<i>Tapirira guianensis</i> Aubl.	4	7	0.35 (0.11)	49.0 (1.31)
	<i>Vochysia tucanorum</i> Mart.	3	7	0.40 (0.06)	49.8 (0.68)
SSF	<i>Aspidosperma polyneuron</i> Müll.Arg.	3	28	0.55 (0.09)	50.1 (3.17)
	<i>Centrolobium tomentosum</i> Guill. ex Benth.	3	7	0.58 (0.14)	50.8 (2.62)
	<i>Croton floribundus</i> Spreng.	2	5	0.35 (0.08)	51.6 (1.87)
	<i>Croton floribundus</i> Spreng.	3	20	0.35 (0.07)	50.0 (2.48)
	<i>Croton floribundus</i> Spreng.	4	6	0.23 (0.03)	50.2 (0.89)
	<i>Croton floribundus</i> Spreng.	5	6	0.22 (0.14)	49.6 (2.32)
	<i>Esenbeckia leiocarpa</i> Engl.	3	5	0.64 (0.10)	49.1 (2.44)
	<i>Inga striata</i> Benth.	3	10	0.26 (0.10)	49.8 (2.26)
	<i>Machaerium brasiliense</i> Vogel	3	5	0.47 (0.06)	47.8 (2.19)
	<i>Ocotea</i> sp.	2	7	0.47 (0.11)	51.3 (2.25)
	<i>Ocotea</i> sp.	3	68	0.53 (0.17)	50.0 (3.05)
	<i>Ocotea</i> sp.	4	25	0.36 (0.15)	50.3 (4.42)
	<i>Ocotea</i> sp.	5	6	0.22 (0.22)	50.5 (7.24)
	<i>Piptadenia gonoacantha</i> (Mart.) J.F.Macbr.	3	19	0.35 (0.11)	48.9 (3.61)
	<i>Piptadenia gonoacantha</i> (Mart.) J.F.Macbr.	4	12	0.22 (0.10)	52.0 (3.34)
	<i>Piptocarpha sellowii</i> (Sch. Bip.) Baker	3	5	0.34 (0.05)	48.3 (1.98)
	<i>Savia dictyocarpa</i> Müll.Arg.	3	10	0.38 (0.12)	47.8 (2.47)
	<i>Savia dictyocarpa</i> Müll.Arg.	4	5	0.43 (0.21)	47.8 (0.99)
	<i>Senegalia polyphylla</i> (DC.) Britton & Rose	3	5	0.43 (0.08)	50.4 (2.88)

5 MODELING BIOMASS AND CARBON OF COARSE WOODY DEBRIS IN TWO FOREST TYPES OF BRAZIL

Abstract

The objective of this study is to improve biomass and carbon stock estimates of coarse woody debris (CWD) in a Seasonal Semi-deciduous Forest (SSF) and in a Cerrado *sensu-stricto* (CSS) area by developing regression models to predict the biomass and carbon of CWD elements using dendrometric variables and decay class. Data were collected using the line intersect sampling (LIS) method. Twenty sampling units were established in both areas using a systematic sampling protocol, with a total 650 m of transect lines per sampling unit. All CWD elements with a diameter ≥ 10 cm that crossed the transect were tallied. For each element, the diameter, length, and decay class was recorded. Disc samples were taken from each element, from which cylinder samples were extracted then oven dried to determine density. These cylinders were milled and analyzed to determine carbon content (%). The volume of each element was calculated by taking the difference between cross-sectional area and the hollowed area, multiplied by the length. Biomass was calculated by multiplying volume values by density values, and carbon stocks were calculated by multiplying biomass by the carbon factor computed via lab analysis. The data from the 650 m of transect lines from each sampling unit were used to fit linear and nonlinear models. Predictor variables included diameter (D_k) and length (L_k), and furthermore a “combined” variable, $c_k = D_k^2 L_k$ was used. All possible combinations of linear models and three different nonlinear models were fitted. Goodness-of-fit was assessed using graphical investigation; furthermore, the *AIC* (Akaike Information Criterion) and the *BIC* (Bayesian Information Criterion) were both calculated. Due the biomass data exhibiting heteroscedasticity, residual variance was modeled as a power function of the combined variable. The best linear model used the combined variable in its original scale with the variance power function. The nonlinear models presented an improvement over the linear ones with smaller values of *AIC* and *BIC*. Using the selected nonlinear model, we added decay class (*DC*) as an indicator (*dummy*) variable, and we also modeled error variance common across decay classes and for each class separately. The best model demonstrated varying intercept values (β_0) for each of the five classes, with β_1 and β_2 common across all classes. Nonlinear models including the variables diameter and length proved an efficient tool for predicting CWD biomass and carbon at the element level. Heteroscedasticity was mitigated by modeling the variance of the residuals with the power function. Adding decay class as an indicator variable also showed model improvement. Common and separately error variance by decay class did not show differences.

Keywords: Coarse woody debris; Allometric equation; Model selection; Biomass; Carbon

5.1 Introduction

Most of the previous research on forest biomass has focused on the living, above ground arbo-real structures (main stem, bole bark, and crown including the branches and leaves), probably because these components of the carbon pool represent the largest fraction of total biomass. Moreover, methods for quantifying these components are simple and usually do not pose many logistical problems. Excluding dead material can lead to an underestimation of the total amount of carbon sequestered in forests (BROWN, 1997). Coarse woody debris (CWD) is a substantial and important component of all forest ecosystems. It provides habitat for many organisms, and is a part of the biogeochemical flows within the forest environment. The large mass of CWD and the prominent role it plays in the global carbon cycle

demonstrates the importance of including CWD in studies of forest biomass and carbon sequestration (CARMONA *et al.*, 2002).

Biomass at the tree level is commonly estimated through the use of regression models. Collecting biomass data can be very labor intensive, with many small samples selected from individual trees by some probabilistic procedure. The green and oven-dry weights are then determined in a laboratory, and the results are used to extrapolate and estimate the biomass for an entire tree. Predictions of biomass of the live above-ground components have commonly been based on tree dimensions including diameter at breast height (D), height (H), D^2H , age, live crown length, diameter at the base of the live crown, and sapwood area. Frequently the residuals from these models exhibit heteroscedasticity, indicating that the error variance is not constant over the range of predictions. Typically, this situation requires some weighting or modeling of the error variance to meet the requirement of uniform error variance (PARRESOL, 2001).

Some modeling studies on coarse woody debris may also have different objectives. RADTKE *et al.* (2009) model CWD biomass as a decay process over time. They predict the dry weight to green volume ratios of single pieces of CWD, in addition to their nitrogen concentration by weight, given observed values of decay and position classes, and latitude. RUSSELL *et al.* (2014) repeated coupled measurements of dead woody debris with models to estimate the duration of various stages of decay. FRAVER *et al.* (2013) present a method for estimating changes in CWD log volume through time and illustrate the method using a chronosequence approach, wherein concurrent changes in wood density from the same logs allow for the quantification of biomass.

The objective of this study is to improve the estimates of the biomass and carbon content of CWD in a Seasonal Semi-deciduous Forest and in a Cerrado *sensu-stricto* ecosystems, by developing regression models to predict the biomass and carbon of coarse woody debris elements from dendrometric variables and decay class.

5.2 Material and methods

5.2.1 Study sites

Data was collected from two different vegetation types, both located in two ecoregions within the São Paulo State, Brazil. In the Brazilian Atlantic Forest, the survey was conducted in a Seasonal Semi-deciduous Forest (SSF), also known as a tropical seasonal forest, located in the Caetetus Ecological Station. The Cerrado ecoregion was located in the Cerrado *sensu-stricto* (CSS) within the Mogi Guaçu Biological Reserve.

The existing forest at the Caetetus Ecological Station constitutes one of the most significant remaining examples of SSF in Brazil. The “semi-deciduous” denomination refers to the seasonally cold weather and the reduction of available water in the soil, together with other environmental factors, that cause the arboreal species to shed much of their leaves in winter, thereby reducing water consumption and decreasing the rate of the plant growth and development (TABANEZ *et al.*, 2005). According to its Management Plan, the Caetetus Ecological Station covers the cities of Galia and Alvinlândia, totaling an area of 2178 ha. The elevation ranges from 520-680 m, and it is dominated by lower slopes (with less than a 6%). It is located in the northern border area of the hydrographic basin of the Paranapanema river. The climate, according to the KÖEPPEN (1948) classification is Cwa: mesothermal dry winter with temperatures below 18° C and above 22° C in summer. In the driest month, total precipitation is only 30 mm; annual precipitation ranges from 1100 to 1700 mm (TABANEZ *et al.*, 2005).

The Mogi-Guaçu Biological Reserve is part of the old Campininha Farm, located in the Martin Prado Junior district of the city of Mogi Guaçu, SP. Totaling 470 ha, it has a relatively flat landscape, with slopes less than 15%. In the areas of Campininha Farm the elevation ranges from 566 to 724 m.

The climate is mesothermal with two well-defined seasons: a dry winter (April to September) and a hot summer (October to March). The average annual rainfall is 1335 mm and the average temperature is $20.5^{\circ}C$ (SÃO PAULO, 2015). The Unit is divided in two parts, one designated as area “A” and the other as area “B”. Area “A”, where this study was performed, totals 343 ha and is dominated by Cerrado ecosystems at different regeneration stages. The main formation is the savanna woodland (spanning pioneer successional stages to advanced), but there are also small patches of herbaceous vegetation near the watercourses.

5.2.2 Data collection

In the field survey, we measured CWD elements that exhibited diameters greater than or equal to 10 cm. Specifically, we used the line intersect sampling (LIS) method, wherein the sample unit is a transect line on which a researcher tallies all downed dead wood following the field protocols of GREGOIRE and VALENTINE (2008). In both study areas, we systematically installed 20 sampling units, with a total of 650 m of transect line length per sampling unit: 200 m in the North-South direction, one line in the East-West direction of 200 m crossing the middle of the N-S transect, another E-W line of 150 m crossing the N-S line 25 m south of the center, and an additional E-W 50 m line crossing the N-S line 50 m south from the center (Figure 5.1).

Figure 5.1: Sampling unit with a transect in the North-South direction (continuous line, 200 m), and three transects in the East-West direction (dashed lines)

The diameter of each element was tallied at the point where the line intersects the element. Two measurements were taken with a caliper with one parallel to the ground and the other perpendicular. If the bark was still attached to the element, the diameter was measured with it. The length of the element was also measured with a tape along its central axis, regardless of the shape. Each element was classified into one of five decomposition stages referenced from HARMON *et al.* (1986) (Table 5.1).

For each element, as well, a sample disc was collected at the intersection point of the line and the element. These samples were taken using a chain saw or a hand saw for elements that had a high degree of decomposition.

To determine the basic density, samples were collected from the discs using the cylinder extraction method proposed by KELLER *et al.* (2004). The extraction of the cylinders was performed in the laboratory with a cylindrical drill of known volume. For elements that were highly decomposed, the cylinders were removed manually using an aluminum cylinder. The cylinders from each disc sample were then oven dried at $65^{\circ}C$ until achieving a constant weight.

Table 5.1: Classes of coarse woody debris decomposition. Adapted from HARMON *et al.* (1986)

Decay class	Definition
1	Woody material consisting of solid wood, with leaves and/or fine twigs attached to the principal part, without noticeable degradation
2	Solid woody material with intact bark, but no leaves or fine twigs
3	Solid wood, material similar to class 2, except with rotting bark
4	Material somewhat rotten, and can be broken when kicked
5	Material that is rotten, friable, and can be broken with bare hands

Sample cylinders from each disc were milled in a knife mill (*Wiley* type), and sieved through a no. 40 mesh, the equipment was vacuum-cleaned between samples to avoid contamination. Measurements of carbon content were performed with *LECO-C632* Carbon Analyzer which uses infrared sensors to detect elemental carbon in the outflow of gas from the combustion system. Results are expressed as percentage of mass. For each sample, two replicates were made to determine final carbon content. All laboratory analyses were performed in the Department of Forest Sciences at the University of São Paulo (ESALQ/USP).

5.2.3 Determination of biomass and carbon

The cross-sectional area of each disc sample from each element was calculated in square meters using the geometric mean diameter, dq_k , of the two diameters measurements taken: $g_k = \pi \left(\frac{dq_k}{40000} \right)^2$.

The volume for each element (v_k) was calculated as the product of the cross-sectional area (g_k) of the k^{th} element multiplied by the length of the k^{th} element. For sample disks with holes or void areas, the cross-sectional area was reduced to account for the holes. These disks were photographed, and the cross-sectional area of the holes was estimated using *ImageJ* software (RASBAND, 2016).

The wood density (ρ_k) of each k^{th} element was determined by computing the average of the sub-sample cylinders. The density of each cylinder was computed as the dry weight (g) divided by fresh volume (cm^3). The biomass (b_k) for each k^{th} element was calculated as the product of its volume and its density: $b_k = v_k \cdot \rho_k$. Finally, the carbon content in each k^{th} element was calculated as the product of its biomass and the average carbon concentration of the subsample of that element determined in the laboratory analysis using the *LECO-C632* equipment.

From the SSF, $n = 551$ elements were measured; from the CSS $n = 209$. All data were processed and analyzed using the R statistical software (R CORE TEAM, 2016). Table 5.2 summarizes the characteristics of the CWD elements measured. The scatterplots of CWD biomass and carbon versus diameter and length for each area are shown in Figures 5.2 and 5.3.

Table 5.2: Summary of CWD elements sampled by forest type. SD is standard deviation, SSF is Seasonal Semi-deciduous Forest, and CSS is Cerrado *sensu-stricto*

		Minimum	Mean	SD	Maximum
SSF n = 551	Biomass (kg)	0.66	114.36	309.40	3728.49
	Carbon (kg)	0.33	57.26	157.09	1992.88
	Diameter (cm)	7.35	18.20	9.25	72.00
	Length (m)	0.40	5.32	4.09	20.20
CSS n = 209	Biomass (kg)	0.54	42.23	84.72	884.50
	Carbon (kg)	0.27	21.12	42.62	444.46
	Diameter (cm)	9.00	15.69	6.19	46.50
	Length (m)	0.30	3.65	2.40	12.75

Figure 5.2: The scatterplots between CWD biomass and diameter (a), CWD biomass and length (b), CWD carbon and diameter (c), and CWD carbon and length (d) for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.3: The scatterplots between CWD biomass and diameter (a), CWD biomass and length (b), CWD carbon and diameter (c), and CWD carbon and length (d) for the Cerrado *sensu-stricto* (CSS)

5.2.4 Modeling

For modeling CWD biomass and carbon of each element, alternative models were fitted by forest type using the dendrometric variables diameter (D_k) and length (L_k). In a preliminary step, all possible combinations of linear models were fitted with one variable, both variables, and with a “combined” variable, $c_k = D_k^2 L_k$. Models using the logarithms of the predictor variables were also evaluated. After testing the linear models, the nonlinear models below were also fitted:

$$y_k = \beta_0 D_k^{\beta_1} L_k^{\beta_2} + \varepsilon_k \quad (5.1)$$

$$y_k = \beta_0 (D_k^2 L_k)^{\beta_1} + \varepsilon_k \quad (5.2)$$

$$y_k = \beta_0 D_k^{\beta_1} + \varepsilon_k \quad (5.3)$$

where y_k is the response variable (biomass or carbon) of the k^{th} CWD element; β_0 , β_1 , and β_2 are the model parameters; and ε_k is the residual error.

Models were fitted with the `gls` and `gnls` functions of the `nlme` package in R (PINHEIRO *et al.*, 2009), for linear and nonlinear models, respectively. The residuals exhibited heteroscedasticity, therefore the residual variance in all models was specified as a power function of the combined (c_k) covariate (PINHEIRO and BATES, 2000): $Var(\varepsilon_k) = \sigma^2 |c_k|^{2\delta}$. Here δ models the heteroscedasticity, and σ acts as a scaling factor for the error variance (GREGOIRE, 2015).

The categorical predictor, decay class (DC), was added to the best selected model as an indicator (i.e., *dummy*) variable. GREGOIRE (2015) presents an explanation of the vocabulary and concepts. These models were refitted to allow an estimate of the error variance separately for each decay class (GREGOIRE, 2015). The variance function structure that allows a different variance for each level of decay class is $Var(\varepsilon_k) = \sigma^2 \delta_{DC_k}^2$ (PINHEIRO and BATES, 2000).

Goodness-of-fit was assessed using various tools. Graphical investigations of the data and scatterplots of model residuals were conducted. We also used the AIC (Akaike Information Criterion) and the BIC (Bayesian Information Criterion). AIC is a widely used model discrimination that penalizes parameter-rich models that estimate the relative general discrepancy between an approximation model and the hypothetical real model; the lower the AIC value of a model, the smaller its relative distance from the hypothetical real model. The BIC penalizes models with more parameters even more than the AIC . Models with smaller values of BIC are also considered better models (BURNHAM and ANDERSON, 2002).

5.3 Results and discussion

Both areas (SSF and CSS) and both response variables (biomass and carbon) presented the same behavior in the model selection process. The best linear model (5.4) used the combined variable in its original scale with the variance power function. However, the nonlinear models presented an improvement over the linear ones. The best nonlinear model was the equation (5.1) that modeled the heteroscedasticity with the variance power function. This model presented the smallest values of AIC and BIC . Table 5.3 shows the linear and nonlinear models parameters estimations for biomass and carbon in both study areas.

$$y_k = \beta_0 + \beta_1 D_k^2 L_k + \varepsilon_k \quad (5.4)$$

Table 5.3: Parameter estimates for the linear model (5.4) and nonlinear model (5.1) fitted and selected for the two response variables (biomass and carbon) to the Seasonal Semi-deciduous Forest (SSF) data and Cerrado *sensu-stricto* (CSS) data. Standard error in parenthesis

$\hat{y}$	Model	$\hat{\delta}$	$\hat{\sigma}$	$\hat{\beta}_0$	$\hat{\beta}_1$	$\hat{\beta}_2$	AIC	BIC
Biomass	Linear SSF	0.9383	0.0203	-0.7649 (0.1992)	0.0313 (0.0001)		4495.75	4512.98
	Nonlinear SSF	0.9275	0.0202	0.0267 (0.0029)	1.9001 (0.0423)	1.2560 (0.0262)	4395.89	4417.45
	Linear CSS	0.9882	0.0143	-0.5828 (0.2582)	0.0310 (0.0012)		1502.73	1516.06
	Nonlinear CSS	0.9801	0.0145	0.0220 (0.0053)	2.0235 (0.0926)	1.2001 (0.0470)	1475.24	1491.95
Carbon	Linear SSF	0.9450	0.0098	-0.3611 (0.0999)	0.0156 (0.0003)		3752.56	3769.79
	Nonlinear SSF	0.9339	0.0098	0.0134 (0.0015)	1.8968 (0.0435)	1.2582 (0.0267)	3651.24	3672.80
	Linear CSS	0.9978	0.0070	-0.2894 (0.1313)	0.0155 (0.0006)		1230.10	1243.43
	Nonlinear CSS	0.9886	0.0071	0.0111 (0.0028)	2.0164 (0.0970)	1.2067 (0.0488)	1200.36	1217.07

Appendices I to IV(a-c) show the models' residuals plotted versus the predictor variables. Appendices I to IV(d) show the residuals plotted versus the fitted values. The graphs show that the residuals are not uniformly arranged along the horizontal axis, which due to the size distribution of the data collected - a concentration of elements with small values of diameter, length, biomass and carbon. To further investigate the model fit, the plots were truncated in the area with small biomass and carbon values, then divided into two x-axis scales so as not to obscure any information (Figures 5.4 and 5.6). These figures show a good distribution of the residuals around zero; i.e., no presence of heteroscedasticity. Note also that the residuals that are largest in absolute value have not interfered with the fit of the model, they were not dropped from the analysis. Although the values of β_1 and β_2 are close to 2 and 1 - the corresponding values in the combined covariate - the goodness-of-fit values indicate that there was an improvement in obtained by including these additional parameters. Figures 5.5 and 5.7 show the fitted curve of the model (5.1).

In the nonlinear models with biomass and carbon, the only parameter that distinguished between them is β_0 . In the carbon models, this parameter is approximately the half the value observed in the biomass models, and this is explained by the fact that carbon is close to 50% of the biomass.

Using the selected nonlinear model, we added decay class (DC) as an indicator variable, using the variance power function to address the heteroscedasticity and examined whether we should use a common error variance or a separate for each class. These models were tested using partially reduced versions, which presumes decay class distinctions for some of the parameters but not all of them. The best model has a different intercept (β_0) for each of the five classes, with β_1 and β_2 common across all classes (5.5).

Figures 5.8 and 5.11 shows the truncated residuals plot of the models. As we observe the models without decay classes, there is no visual sign of variance heterogeneity. Table 5.4 shows the estimation of these models fitted to SSF and CSS data. The results show approximately identical values in all respects fitting models with common and separately error variance for decay classes. So, the presumption of a common error variance can be maintained. Figures 5.9, 5.10, 5.12, and 5.13 show the fitted curve of the model (5.5) by decay class.

In Tables 5.3 and 5.4, the estimated parameters between SSF and CSS forest types appear to be similar; however as these forest types are very different ecosystems, separate models are presented for each forest type in this study.

$$y_k = \beta_{01} DC_1 D_k^{\beta_1} L_k^{\beta_2} + \beta_{02} DC_2 D_k^{\beta_1} L_k^{\beta_2} + \beta_{03} DC_3 D_k^{\beta_1} L_k^{\beta_2} + \beta_{04} DC_4 D_k^{\beta_1} L_k^{\beta_2} + \beta_{05} DC_5 D_k^{\beta_1} L_k^{\beta_2} + \varepsilon_k \quad (5.5)$$

Table 5.4: Parameter estimates for the nonlinear models with decay class as an indicator variable (5.5) fitted with the two response variables (biomass and carbon) to the Seasonal Semi-deciduous Forest (SSF) data and Cerrado *sensu-stricto* (CSS) data. Standard error is in parentheses

y	Model	$\hat{\delta}$	$\hat{\sigma}$	$\hat{\beta}_0(DC1)$	$\hat{\beta}_0(DC2)$	$\hat{\beta}_0(DC3)$	$\hat{\beta}_0(DC4)$	$\hat{\beta}_0(DC5)$	$\hat{\beta}_1$	$\hat{\beta}_2$	AIC	BIC
Biomass	Nonlinear SSF ¹	0.9730	0.0134	0.0406 (0.0050)	0.0375 (0.0042)	0.0348 (0.0037)	0.0242 (0.0026)	0.0152 (0.0025)	1.9281 (0.0407)	1.1040 (0.0259)	4297.46	4336.26
	Nonlinear SSF ²			0.0370 (0.0048)	0.0346 (0.0038)	0.0319 (0.0033)	0.0226 (0.0024)	0.0144 (0.0019)	1.9475 (0.0398)	1.1194 (0.0268)	4281.90	4337.95
	Nonlinear CSS ¹	0.9958	0.0123	0.0381 (0.0103)	0.0282 (0.0071)	0.0282 (0.0065)	0.0200 (0.0047)	0.0161 (0.0045)	2.0170 (0.0876)	1.0935 (0.0476)	1451.42	1481.50
	Nonlinear CSS ²			0.0404 (0.0093)	0.0301 (0.0072)	0.0300 (0.0068)	0.0210 (0.0048)	0.0167 (0.0039)	2.0038 (0.0851)	1.0765 (0.0471)	1444.84	1488.29
Carbon	Nonlinear SSF ¹	0.9737	0.0068	0.0203 (0.0025)	0.0190 (0.0022)	0.0171 (0.0018)	0.0120 (0.0013)	0.0074 (0.0012)	1.9320 (0.0415)	1.1037 (0.0265)	3555.09	3593.90
	Nonlinear SSF ²			0.0186 (0.0024)	0.0176 (0.0019)	0.0157 (0.0017)	0.0113 (0.0012)	0.0070 (0.0010)	1.9511 (0.0406)	1.1180 (0.0273)	3542.06	3598.11
	Nonlinear CSS ¹	1.0094	0.0059	0.0198 (0.0056)	0.0147 (0.0039)	0.0147 (0.0036)	0.0104 (0.0026)	0.0080 (0.0024)	2.0055 (0.0926)	1.0870 (0.0493)	1177.56	1207.64
	Nonlinear CSS ²			0.0200 (0.0048)	0.0149 (0.0037)	0.0150 (0.0035)	0.0105 (0.0025)	0.0080 (0.0020)	2.0026 (0.0886)	1.0817 (0.0487)	1169.55	1213.00

¹ Common δ and σ parameters for all decay classes

² Separate δ and σ parameters for each decay class

Figure 5.4: The truncated scatterplot between the standardized residuals of the model (5.1) vs. the fitted values of the model from biomass data, ranging from 0 to 100 kg (a) and 100 to 3800 kg (b), and from carbon data, ranging from 0 to 50 kg (c) and 50 to 2000 kg (d) for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.5: Biomass versus combined variable($D^2 L$) and fitted curve of the model (5.1) (a) Carbon versus combined variable($D^2 L$) and fitted curve of the model (5.1) (b) for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.6: The truncated scatterplot between the standardized residuals of the model (5.1) vs. the fitted values of the model from biomass data, ranging from 0 to 60 kg (a) and 100 to 885 kg (b), and from carbon data, ranging from 0 to 20 kg (c) and 20 to 445 kg (d) for the Cerrado *sensu-stricto* (CSS)

Figure 5.7: Biomass versus combined variable($D^2 L$) and fitted curve of the model (5.1) (a) Carbon versus combined variable($D^2 L$) and fitted curve of the model (5.1) (b) for the Cerrado *sensu-stricto* (CSS)

Figure 5.8: The truncated scatterplot between the standardized residuals of the model (5.5) vs. the fitted values of the model by decay class (DC), with biomass values ranging from 0 to 100 kg (a) and 100 to 3800 kg (b), and with carbon values ranging from 0 to 50 kg (c) and 50 to 2000 kg (d) for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.9: Biomass versus combined variable($D^2 L$) and fitted curve of the model (5.5) by decay class for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.10: Carbon versus combined variable($D^2 L$) and fitted curve of the model (5.5) by decay class for the Seasonal Semi-deciduous Forest (SSF)

Figure 5.11: The truncated scatterplot between the standardized residuals of the model (5.5) vs. the fitted values of the model by decay class (DC), with biomass ranging from 0 to 60 kg (a) and 100 to 885 kg (b), and with carbon values ranging from 0 to 20 kg (c) and 20 to 445 kg (d) for the Cerrado *sensu-stricto* (CSS)

(a)

Figure 5.12: Biomass versus combined variable($D^2 L$) and fitted curve of the model (5.5) by decay class for the Cerrado *sensu-stricto* (CSS)

Figure 5.13: Carbon versus combined variable($D^2 L$) and fitted curve of the model (5.5) by decay class for the Cerrado *sensu-stricto* (CSS)

5.4 Conclusion

Nonlinear models that include the variables diameter and length proved an efficient tool for predicting CWD biomass and carbon for individual elements. As expected, biomass and carbon data exhibited heteroscedasticity, which was mitigated by modeling the variance of the residuals with a power function of the combined ($D^2 L$) variable.

Adding decay class as an indicator variable to the model also showed improvement in goodness of fit, especially considering the evaluation of the characteristics when applying the model. The biomass and carbon data from both forest types, SSF and CSS, exhibited the same behavior for the model selection process. More studies should be made to conclude if the same model can be used for both ecosystems or whether separate equations are required. In conclusion, CWD is an important forest component, and more studies are needed to improve the calibration and analyses of allometric equations for predicting the biomass of CWD.

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Appendices

Appendix I - The scatterplots between the standardized residuals of the model (5.1) of biomass vs. predictor variables: diameter (a), length (b), combined variable (c); and the standardized residuals vs. the fitted values (d) for biomass of Seasonal Semi-deciduous Forest (SSF)

Appendix II - The scatterplots between the standardized residuals of the model (5.1) of biomass vs. predictor variables: diameter (a), length (b), combined variable (c); and the standardized residuals vs. the fitted values (d) for biomass of Cerrado *sensu-stricto* (CSS)

Appendix III - The scatterplots between the standardized residuals of the model (5.1) of carbon vs. predictor variables: diameter (a), length (b), combined variable (c); and the standardized residuals vs. the fitted values (d) for carbon of Seasonal Semi-deciduous Forest (SSF)

(a)

(b)

(c)

(d)

Appendix IV - The scatterplots between the standardized residuals of the model (5.1) of carbon vs. predictor variables: diameter (a), length (b), combined variable (c); and the standardized residuals vs. the fitted values (d) for carbon of Cerrado *sensu-stricto* (CSS)