***Senna reticulata*: a viable option for bioenergy production in the Amazonian region**

Adriana Grandis1\*, Bruna C. Arenque-Musa1\*, Marina C. M. Martins1, Thais Olivar Maciel1, Rachael Simister2, Leonardo D. Gómez2, and Marcos S. Buckeridge1\*\*

1 Laboratório de Fisiologia Ecológica de Plantas, Department of Botany, University of São Paulo, Brazil

2 Centre for Novel Agricultural Products, Department of Biology, University of York, England

\* These authors contributed equally to this work

\*\* Correspondence author: tel +55 (11) 30917592, fax +55 (11) 30917547; email: [msbuck@usp.br](mailto:msbuck@usp.br)

**Abstract**

*Senna reticulata* is an Amazonian tree that quickly accumulates high biomass. It grows widely in the North of Brazil occupying degraded regions and is popularly known as “matapasto” (pasture-killer) due to its aggressive colonization strategy. When its aerial parts are harvested, *S. reticulata* recolonizes the pasture quickly recovering biomass production. In this work, we examined the potential of *S. reticulata* for bioenergy production in the Amazon region and the effect of a CO2 enriched atmosphere on its biomass composition. Nearly 50% of the biomass of the aerial parts is non-structural carbohydrates (NSC). Concerning structural carbohydrates, pectins (25% and 23%), hemicelluloses (11% and 16%), and cellulose (4% and 14%) contents were very similar in leaves and stems, respectively. Lignin varied considerably among organs, being 35% in roots, 7% in stems, and 10% in leaves. Although elevated CO2 did not change significant cell wall pools, lignin content was reduced in leaves and roots. Furthermore, starch increased 31% in leaves under elevated CO2, which improved saccharification by 47%. We conclude that *Senna reticulata* is a suitable species for use as a bioenergy feedstock in the tropics and specifically for remote communities in the Amazonian region.

**Keywords:** Starch, saccharification, climate change, ethanol, CO2, bioenergy.

**Introduction**

Biofuels, such as bioethanol and biodiesel, have been accounted for as solutions to help mitigation and adaptation to global climate change in the transportation sector [1]. First-generation (1G) bioethanol is usually made of sugarcane (*Saccharum* spp.) and sugar beet (*Beta vulgaris*) following sucrose extraction and fermentation. Crops like maize (*Zea mays*) are also used for bioethanol production, requiring the action of starch degrading enzymes before glucose fermentation [2, 3]. Alternatively, lignocellulosic biomass (from agricultural residues, trees like poplar, and other non-food crop species) can also be used as feedstock for second-generation (2G) ethanol production. This can be or not complementary to 1G depending on the feedstock and involves the physical breaking of biomass material, pretreatment, and separation of liquid and solid components [4]. One beneficial aspect of the use of 2G crops (like willow and energy-cane) is their cultivation in marginal lands, which decreases the pressure on food and feed production [5].

2G processes are limited by the complexity of structural carbohydrates (the polymers present in cell walls) so that pretreatment and hydrolysis with enzymes able to degrade cellulose and hemicelluloses are required to release fermentable sugars [3, 6]. Additionally, lignin represents not only a structural barrier to cell wall hydrolysis but is also a limiting factor for high solid loading that decreases ethanol titer [7, 8].

Brazil is the second worldwide ethanol producer based on commercial sugarcane cultivars [5]. However, sugarcane cultivation is limited to few highly-productive regions (especially southeast region), and ethanol price becomes elevated with the distance between producers and consumers [9]. To overcome this limitation and promote the development of remote regions of Brazil, such as the urbanized areas and settlements of the Amazon, it would be essential to facilitate the use of native species as alternative feedstocks for biofuel production. These species could be grown in forest degraded areas to improve restoration and carbon sequestration, making it possible sustainable energy generation. One successful example is the utilization of palm oil as a source of biodiesel in the Amazon [10]. The Amazon region represents the most substantial amount of communities living in remote areas (e.g., islands or distant locations usually separated by wide rivers), which are generally small-scattered population groups (100 to 500 inhabitants) with weak or nonexistent work conditions [10]. Their survival depends on hunting, fishing, family agriculture, and forest exploitation, and their homes lack electricity that cannot be economically and technically supplied by conventional systems. These communities could gain advantage of the forest biomass with good potential to generate sufficient electrical power to demand supply [10].

The Amazon Forest has exceptional plant species diversity [11] that is still unexploited for bioenergy purposes. In this work, we propose *Senna reticulata* Irwin and Barneby (Leguminosae) as a target species for 1G and 2G bioethanol and electricity production in Amazon regions. *S. reticulata* is a fast-growing pioneer tree with high photosynthetic capacity widely spread in nutrient-enriched soils (especially along rivers – “Whitewater rivers”), considered one of the most productive trees of the Amazon floodplains [12, 13]. Seedlings of *S. reticulata* show an increase of 4 meters in height after 8 months [14], which in adult individuals corresponds to 52 kg of fresh shoot biomass [12]. When comparing it with *Salix humboldtiana* and *Cecropia latiloba*, other native pioneer species of Amazon that form monospecific stands with similar characteristics linked to the habitat conditions, *S. reticulata* has significant advantages such as 50 cm stem elongation per month in contrast to 10 cm in these other two species [15]. *S. reticulata* has a high tolerance to more extended flooded periods, high resprout capacity and intense lateral growth that favor the competition for space and light [14] and make it even more productive in its subsequent years after establishment. This species has several adaptations to survive periodical waterlogging, such as adventitious roots, lenticels, and leaf size adjustments [16]. Furthermore, *S. reticulata* has also a very efficient strategy of “escape from submergence” [17] characterized by investment in growth leading to high shoot elongation and leaf protrusion above the water surface. These features collectively make local people in Amazon consider it as a woody weed (“matapasto” or “pasture killer”). However, *S. reticulata* can also enhance the establishment of other species in degraded environments due to its shade and humidity as a result of its fast-growing pattern [12]. Here, we characterized the non-structural (NSC) and structural (SC) carbohydrates from *S. reticulata*, the impact of increased atmospheric CO2 on their levels, as well as its potential for biomass saccharification to improve bioenergy resources for remote communities in Amazon rainforest.

**Material and methods**

*Plant material*

Seeds of *Senna reticulata* (Figure 1A-E) were obtained from a field site in Belém-PA (Brazil), germinated and cultivated as described in Arenque et al. [13]. Control and treated plants were kept in open-top chambers (OTC’s) with ambient atmospheric CO2 (380 µmol.mol–1) or elevated CO2 (760 µmol.mol–1), respectively. The experiment was conducted from January to April 2009 under a natural photoperiod (summer-autumn in the Southern Hemisphere - 13.5 h light and 12.5 h dark). After 90 days, 5 plants were collected from each treatment for cell wall biochemical analyses.

Plants were separated into leaves, stems, and roots and immediately frozen in liquid nitrogen, lyophilized, subsequently ground in a ball mill, and stored until biochemical analysis.

*Cell wall fractionation*

Soluble sugars were extracted exhaustively from 500 mg of powdered material in 80% ethanol (80 °C) for 20 min. This procedure repeated four times. The starch was extracted with 25 mL of 90% DMSO (dimethylsulfoxide), and the suspension stirred vigorously for 12 h [18]. The following day, the starch extraction was repeated three times (3 h) and washed extensively with distilled water. The remaining pellets are composed of SC and were used for cell wall fractionation, as described by De Souza et al. [19].

Pectins were removed by three consecutive incubations with ammonium oxalate 0.5% (OX) per 1 h at 80 °C each. After centrifugation, the supernatants were pooled. Depectinated samples were washed with distilled water and transferred to 40 mL of 3% sodium chlorite in 0.3% acetic acid for 2 h at 80 °C [20]. After centrifugation, supernatants were pooled, whereas the pellets were washed with distilled water five times before the addition of NaOH.

Graded solutions of NaOH (0.1 M, 1 M, and 4 M) with NaBH4 (3 mg. mL-1) were used to extract hemicelluloses for 1 h at room temperature, this procedure repeated three times. For each NaOH concentration, the supernatants were neutralized with glacial acetic acid.

Supernatants from all cell wall fractions were dialyzed for 48 h in distilled water, lyophilized, and weighed.

Crystalline cellulose was determined after the digestion of non-cellulosic polymers in acetic-nitric acid for 1 h in a boiling water bath [21]. The cellulose residues were washed several times in water, lyophilized, and weighed.

*Monosaccharide analysis*

Two mg of each cell wall fraction was hydrolyzed with 100 µL of 72% H2SO4 (v/v) for 45 min at 30°C. After diluting the acid to 4%, the material was autoclaved for 1 h at 121°C [22]. Samples were neutralized with NaOH and applied to sequential cation/anion exchange columns (Dowex). The neutral monosaccharide composition was determined by High-Performance Anion Exchange Chromatography with a Pulsed Amperometric Detection (HPAEC/PAD) in Thermo-Dionex DX-500 system using a CarboPac PA1 column. Monosaccharides were separated isocratically in 0.8% 150 mM NaOH and water at a flow rate of 1mL.min-1, and detected with a post-column containing 500 mM NaOH at a flow rate of 0.5 mL.min-1. Cell wall monosaccharides (glucose, mannose, arabinose, fucose, rhamnose, galactose, and xylose) were identified and quantified by comparison with original standards.

*Determination of uronic acids*

Uronic acid content of each cell wall fraction was determined by colorimetric assay using the m-hydroxybiphenyl method, using galacturonic acid as standard. Paired reaction reductions obtained the proportions of esterified and unesterified uronic acid components with NaBD4 and NaBH4, respectively [23]. Uronic acids were detected by a carbazole-sulfamate assay [24], and their contents expressed in µg. mg-1 of cell wall fractionation yield.

*Quantification of lignin*

Powdered material (30 mg) was homogenized in 50 mM potassium phosphate buffer pH 7.0. After centrifugation (1,400 *g*/ 4 min), the pellets were successively washed (1 mL) as follows: 2× with potassium phosphate buffer pH 7.0, 3× with 1% (v/v) Triton® X-100 in potassium phosphate buffer pH 7.0, 2× with 1 M NaCl in potassium phosphate buffer pH 7.0, 2×with distilled water, and 2× with acetone. The remaining pellet was dried in an oven (60 °C, 24 h) and cooled in a vacuum desiccator before transference to screw-cap centrifuge tubes containing 1.2 mL of thioglycolic acid and 6 mL of 2 M HCl. Samples were heated at 95 °C, 4 h, and after cooling at room temperature, were centrifuged (1,400 *g*/ 5 min), and supernatants discarded. The pellets containing the complex lignin–thioglycolic acid (LTGA) were washed three times with distilled water and the LTGA extracted by shaking (30 °C, 18 h) in 0.5 M NaOH. After centrifugation (1,400 *g*/ 5 min), the supernatant was stored. The pellets were rewashed with 0.5 M NaOH, and supernatants from the same samples were pooled. The combined alkali extracts were acidified with concentrated HCl. After precipitation (0 °C, 4 h), LTGA was recovered by centrifugation (1,400 *g*/ 5 min) and washed twice with distilled water. Finally, the pellets were dried at 60 °C, dissolved in 0.5 M NaOH, and diluted to yield an appropriate absorbance for spectrophotometric determination at 280 nm [25]. Lignin was expressed as mg LTGA per g of dry weight (DW) [26].

*Saccharification assay*

Powdered material (leaf, stem, and roots) was pretreated with 0.5 N NaOH (cell wall components only) or water (starch and cell wall components), at 90 °C for 30 min. After this, the remaining biomass was rinsed six times with 500 μl of 25 mM sodium acetate buffer before the enzymatic hydrolysis. Samples were incubated with shaking at 50 °C in the presence of an enzyme cocktail described in Gómez et al. [27]. At the indicated times, aliquots were taken from the 96-well plates. The determination of sugars released from saccharification was performed using 3-methyl-2-benzothiazolinone hydrozone (MTBH). The reaction contained 0.1 N NaOH, 0.0215 mg MTBH, and 0.007 mg dithiothreitol (DTT). Samples were incubated at 60 °C for 20 min and subsequently 100 μl of oxidising reagent (0.2% FeNH4(SO4)2, 0.2% sulfamic acid, and 0.1% HCl) was added. Glucose quantification was determined spectrophotometrically at 620 nm using a standard curve (50, 100, and 150 nmols of glucose). The entire procedure was automatically performed by the liquid handling station [27].

*Data analysis*

The cell wall fractionation yields were quantified relative to total biomass, and the total cell wall content. The hexose: pentose ratios were calculated with the sum of all hexoses divided by the sum of the pentoses present in the same extract.

The statistical analysis was performed using the JMP® Statistical Discovery Software (version 5.0.1, SAS Inc., Cary, NC, USA) with Student’s *t*-test for comparison between ambient and elevated CO2 treatments. ANOVA-test and a posteriori contrasts by Tukey range tests were also applied to analyze differences between leaves, stems, and roots. All the tests used significance differences with *P* ≤ 0.05 with five biological replicates per treatment and ten replicates for comparison between plant organs (Supplemental Table 1).

**Results**

*Cell wall composition of* S. reticulata *is only mildly affected by elevated CO2*

NSC represent the main fraction of total biomass in *S. reticulata* leaves (~60%), followed by pectins (OX and CL fractions) (~25%), hemicelluloses (all NaOH fractions) (~11%), and cellulose (residue) (~4%) (Table 1). The high NSC proportion is also observed in other organs (37.5% in stems and 35% in roots), whereas the content of other cell components differs significantly (Suppl. Table 1). Concerning the total biomass, Hemicelluloses represent 23% and 16%, pectins 17% and 19%, and cellulose 14% and 11%, in stem and roots, respectively (Suppl. Table 1).

When only insoluble cell wall fractions were considered, pectins (about 27%), hemicelluloses (about 36%), and cellulose (22%) were equally represented in roots and stems. In leaves, pectins represented 60%, hemicellulose 28%, and cellulose 10% (Table 1 – Suppl. Table 1). The lignin content is quite discrepant among organs, reaching a 5-fold increase in roots (35%) in comparison to stems (7%) and leaves (10%) (Figure 2A).

Although a previous study from our group confirmed that elevated CO2 significantly increased total biomass in *S. reticulata* by 30%[13], our results indicate that this treatment brings about only modest effects in leaves when considering the main cell wall polysaccharide pools. The starch level has risen with elevated CO2, whereas cell wall yield in the CL fraction decreased (Table 1). Furthermore, reduced lignin content was observed in leaves and roots (Figure 2A). At least for leaves, this reduction is in agreement with the low CL yield as lignin is firmly bound to the sugars in this fraction [20, 28]. Cell wall pectins are, theoretically, mainly solubilized in OX, but also in CL fraction, which could also contain more soluble hemicelluloses. OX fractions from all organs presented similar rhamnose contents, while fucose and xylose levels were higher in roots (Tables 2-5). Treatment with elevated CO2 decreased fucose and rhamnose in leaves but increased galactose in roots. Monosaccharide composition revealed that rhamnose levels in CL fraction are also similar among organs, whereas fucose, arabinose, and mannose are more abundant in roots.

Interestingly, several monosaccharides from these more soluble fractions were present at similar levels in leaves and stems, suggesting similar pectin composition in those two organs (Tables 2 and 3). Both OX and CL fractions from roots contain more galactose and arabinose, that together with the overall higher monosaccharide levels, could indicate the presence of branched arabinogalactans (Table 5). Furthermore, glucose content was also shown to be high in those fractions in leaves and stems (Tables 3 and 4), suggesting the presence of some contamination with starch in these fractions. Elevated CO2 has minimal impact on CL composition, altering only mannose in leaves (Table 3).

In the 0.1 M NaOH fraction, glucose and xylose were in higher proportion than the other monosaccharides, possibly indicating the abundance of the hemicellulose xyloglucan even though their proportion differs among organs (Table 2). Elevated CO2 brought about increased xylose and reduced glucose levels in leaves and stems, which may indicate changes in the xyloglucan structure (Tables 3-5). To extract polysaccharides that are tightly bound to cellulose, the remaining insoluble wall residues were treated with higher NaOH concentrations. It is possible to note that the proportions of xylose and glucose remain high in 1 M and 4 M NaOH fractions. However, the xylose content gradually decreases from 0.1 M onwards, suggesting less the presence of less branched xyloglucans. The higher glucose levels in 4 M fraction (73% to 84%) further indicate that xyloglucan is firmly bound to cellulose. No alteration in monosaccharide composition was observed under elevated CO2 (Tables 3-5). The remaining residue fraction is composed basically of cellulose (Table 2).

Uronic acids, significant components of pectins, were shown to be present in significant proportions in most fractions, but in higher proportions in OX (approximately 430 to 480 μg per microgram of fraction) (Tables 2-5).

*Starch content improves saccharification with hot water*

Considering the two pretreatments employed (alkaline and hot water), the saccharification levels were higher for leaves and stems in comparison to roots (Figures 2B, C). However, the saccharification efficiency was lower under alkaline pretreatment (Figures 2B, C). A plausible explanation for this difference is the maintenance of starch content under hot water pretreatment. Indeed, the presence of relatively high levels of glucose in the alkali fractions (attributed to the presence of starch – see above) observed in Tables 2,3, and 4, corroborates these findings. It is possible that the portion of alkali-extractable starch would have been solubilized with alkali, granting the significant difference observed in the saccharification levels of the biomasses under hot water an alkali (Figures 2B, C).

When all organs are analyzed together, lignin was negatively correlated to saccharification levels (R = -0.938) (Figure 2D). Elevated CO2 only affected saccharification in leaves treated with hot water. This is explained by the increased starch levels in the organ (Table 1 and Figure 2C). Indeed, starch content and saccharification with hot water were positively correlated (R = 0.772, *P* = 0.000), (Figure 2E).

**Discussion**

*Cell wall composition of S. reticulata*

The proportions among the main components of the cell walls of *S. reticulata* [pectin (35%), hemicellulose (30%), and cellulose (20-30%)] are consistent with the presence of Type I cell walls [29, 30]. Those proportions were relatively conserved in roots and stems of *S. reticulata.* However,leaves displayed higher pectin content(ca. 60% of the insoluble cell wall fraction) and consequently lower cellulose (10%) and hemicellulose (28%) (Table 1 - Suppl. Table 1). The higher proportion of pectin in leaves might be related to the higher abundance of cells containing primary cell walls. This contrasts with the higher abundance of secondary walls due to the presence of higher proportions of vascular tissues in stem and roots.

Monosaccharides and uronic acids typical from pectins were mainly obtained in OX fraction from leaves and stems of all analyzed organs followed by CL and 0.1 M NaOH but were also observed in the residue (Tables 2-5). Similar to what has been observed in poplar and three Eucalyptus species (*E. globulus*, *E. grandis*, and *E. urophylla*), OX, and sodium carbonate fractions resulted in higher pectin content in comparison to other fractions [31, 32].

The levels of uronic acids, arabinose, and mannose in the residue fraction suggest that insoluble pectin is found tightly associated with cellulose. Evidence that pectin and also pectic/arabinogalactan can be found in all fractions isolated from cell walls, including those often referred to as largely hemicellulose fractions (e.g., from 1 to 4 M KOH fractions) are commonly reported in the literature [31, 33–36]. Additionally, high proportions of galactose and arabinose in roots indicate the presence of more branched arabinogalactans in this organ in comparison to leaves and stems (Tables 2 and 5).

*S. reticulata* showed increased levels of xylose in 0.1 and 1 M NaOH fractions in all organs, which is similar to poplar, a species that display abundant xylan in 1 M KOH extract [31]. Monosaccharide profiles (Table 2) showed that leaves and stems have more similarities in cell wall compounds than roots. A clear difference is related to more branched sugars (pectins and soluble carbohydrates) in roots, such as arabinose and galactose. Moreover, leaves and stems have higher xylose and glucose that possibly correspond to xylan, a carbohydrate from the secondary cell wall [37].

The lignin content represents the main contrast among organs, being highly abundant in roots (35% of DW) (Figure 2A). Species from flooded areas have high concentrations of lignin and/or suberin, especially in the roots, to minimize the damage caused by hypoxia during waterlogging periods [38, 39]. The lignin increases the tissue strength and promotes a hydrophobic barrier to protect against toxic components present in waterlogged soils in anoxic conditions [40, 41].

*Effect of elevated CO2 in S. reticulata cell wall*

Plant growth and biomass accumulation are stimulated under elevated CO2 in several plant species [42–45], including *S. reticulata* [13]. Therefore, in this work, we have investigated whether differences in NSC (e.g., starch and soluble sugars) or structural components could be noticed under this treatment (Table 1). A study conducted with 27 C3 species, including crops, herbaceous, and trees, concluded that the effective change in chemical composition due to elevated CO2 was the accumulation of total NSC (mainly starch) [46]. Corroborating this finding, *S. reticulata* had also shown a significant increase in starch content in leaves (11%) (Table 1). Still, an increment of 6% in total biomass was observed when starch and soluble sugars were not considered in the calculations because these NSC are more abundant in ambient when compared to elevated CO2 treatment (Table 1). Our results show that when comparing only major cell wall carbohydrate pools, no differences in biochemical composition of leaves, stems, and roots are noted under elevated CO2.

Cell wall fractionation did not show significant differences between treatments, indicating that changes contributing to increased biomass probably had occurred more in terms of cell number (more divisions) than in cell wall thickness [47]. Elevated CO2 has been demonstrated to promote an increase in cell size and/or number [48, 49] and stimulate primary growth of shoots by boosting the proportion of rapidly dividing cells and shortening cell cycle duration in shoot apices [50].

Although no changes were found in cellulose or hemicellulose yield fractions (Table 1), lignin content was strongly decreased in leaves and roots under elevated CO2 (Figure 2A). This finding corroborates the idea that more cell divisions would probably be related to the formation of proportionally higher amounts of cells with primary walls. Also, as a whole, tissues would possess weaker cell walls, which might be associated with radial oxygen losses in anaerobic conditions [51, 52].

Due to the instability that elevated CO2 promotes on primary and secondary metabolism [45], it is reasonable to expect that polysaccharides would present modifications in their structure as suggested by increased activity of UDP-Glc-dehydrogenase, a key enzyme in the nucleotide-sugar conversion pathway necessary for the biosynthesis of many cell wall polysaccharides [49, 53]. Modifications in the proportions of monosaccharides from pectins and hemicelluloses fractions were observed (Tables 2-4). At elevated CO2, OX fraction had lower rhamnose and fucose in leaves and galactose in roots. These results possibly represent a significant change in pectin structure in *S. reticulata* plants grown under elevated CO2. An increase in xylose in 0.1 M NaOH in leaves could indicate that structural changes in hemicelluloses were also happening, probably in xyloglucan. Elevated CO2 can improve cell wall extensibility and stimulate cell expansion rates of roots [54] and leaves [55], possibly by the increased activity of xyloglucan endotransglucosylase/hydrolase (XTH) [56]. Together, these results suggest that the effect of elevated CO2 on the cell wall properties is not likely to be reflected in the biomass content *per se*. However, reduced lignin content may have influenced some of the chemical bonds among the polysaccharides, resulting in the few alterations mentioned above. Changes in polymer structures within the walls may promote alterations in their physical properties, such as hardness, extensibility, and recalcitrance that could play a crucial role in the long-term development and consequently in its potential for bioenergy production.

*Potential for bioenergy generation*

The identification and characterization of additional plant species that may be useful as bioenergy feedstock are relevant because they intensify the efforts to mitigate climate changes [57, 58]. It is estimated that 33% of native vascular plants worldwide are found in the Americas, with Brazil having the most diverse flora [59]. Therefore, potential feedstock candidates could be more likely to be discovered among Brazilian species. Due to its vast territory, species from various biomes should be screened, including the Amazon region, where abundant biodiversity occurs.

Currently, the monocot grasses miscanthus, maize, sugarcane, and switchgrass, which have cell wall type II, are grown as bioenergy feedstocks for lignocellulosic biomass production [60–62]. Because *S. reticulata* is a tree species with cell wall type I, comparisons among different woody species such as Pinus and Eucalyptus, also proposed as future biofuel feedstocks [32, 63, 64], are more appropriate. Tree species produce an extensive amount of wood through cambial growth in their stems with similar wood composition and high recalcitrance.

To be considered an adequate source of biomass for bioenergy, a plant species would have to display features such as fast growth, high biomass production, high capacity of accumulation of carbon storage compounds such as cell walls, starch, soluble sugars, and lipids. As a source of carbon, the cell walls are by far the most abundant accumulation of compounds that could be sources of fermentable substances. However, a significant obstacle to the conversion of cellulosic biomass into bioproducts is its recalcitrance to hydrolysis, which prevents access to the cell wall by biological and chemical agents.

It is well established that lignin removal is a crucial step for obtaining greater access to cell wall digestion. Re-engineering lignin biosynthesis or its interactions with the wall polysaccharides has been an important goal [65–69], but information about how lignin and polysaccharides are packed in secondary cell walls at the molecular level is still being elucidated [37, 60]. Here we have demonstrated for *S. reticulata* that lignin and saccharification are negatively correlated. Also, leaves and stems displayed improved saccharification rates in comparison to roots (Fig 2D). These results further corroborate the hypothesis that the rate of enzymatic hydrolysis of cellulose to sugars is inversely proportional to the amount of lignin [37, 61]. Predictions estimate that a feedstock with 22% of lignin would yield only half the sugar of a feedstock with 17%. More dramatically, a feedstock with 26% of lignin would yield almost no sugar [57]. These data suggest that by this criterion herbaceous are more appropriate for bioconversion than woody species because the percentage of lignin in bamboo (25%) and poplar (22%) is substantially higher than switchgrass (17%), *Miscanthus* (17%), and alfalfa (17%) [72, 73]. Lignin contents in stems (7%) and leaves (10%) (Figure 2A) of *S. reticulata* are relatively lower than the feedstocks mentioned above, and together with the fact that saccharification could be increased by almost 50%, it seems that this species is likely to be very useful for bioethanol production.

Furthermore, its leaves and stems can be manually cut easily, after what the plant will quickly resprout. The roots are the most lignified tissue and not very useful for saccharification. It is essential to highlight that although the lignin content was reduced in leaves (50%) and roots (20%) when the plants were grown under elevated CO2, those changes did not increase saccharification (Figure 2A,B).

Not only lignin is a limiting factor for the saccharification, but the composition of biomass is also essential for bioethanol production. A significant increase in saccharification (around 100%) in hot water was obtained in comparison to alkaline pretreatment. This result indicates that the starch levels in the tissue significantly improved saccharification rates (Figure 2B,C). Starch was higher in leaves after treatment with elevated CO2, which also boosted saccharification. Starch and saccharification were positively correlated (Figure 2E). Thus, the rising CO2 concentrations in the atmosphere have the potential to increase saccharification in leaves of *S. reticulata* in the future.

Among the existing alternatives to take reliable electricity supply to remote communities in the Amazon region, small diesel generators are the ones mostly employed, even though they exhibit severe restrictions related to operating conditions due to frequent energy interruptions (working for maximum 4 to 6 hours per day). Those diesel generators also present a poor power quality performance concerning frequency and voltage regulations along with high maintenance costs [10]. However, most of these isolated communities are located in areas that present some renewable resources, such as biomass, with good potential to generate sufficient electrical power. One example is the tropical tree *Leucaena leucocephala* that can produce approximately 35 t of biomass/ha, much superior to hybrid poplar [74], and support annual harvests very useful for rotation coppice [72]. The biomass of *S. reticulata* could be equally suggested to be used for bioelectricity production in remote regions of the Amazon.

**Conclusions**

The Amazonian native species *Senna reticulata* has been shown to display the necessary features to be used as a feedstock for bioenergy production. It is a fast-growing plant, which produces a significant amount of biomass, being suitable for use either as a crop (if adequately domesticated) or as a source of biomass to be used *in natura* by small and remote communities in the Amazon, where its biomass could be used for bioelectricity production in gasifiers. Saccharification of its biomass is favored by the presence of relatively high amounts of non-structural carbohydrates (starch and soluble sugars), which are ideal for fermentation and production of bioethanol. The cell wall composition also benefits from the lower amount of lignin, which favors the process of second-generation bioethanol production. The future use of *S. reticulata* for bioenergy production will probably not be affected since the cell wall composition does not show significant changes under the elevation of atmospheric CO2. On the contrary, the elevation of this greenhouse gas led to faster growth, higher production of biomass, and higher accumulation of non-structural carbohydrates. Together, all these features are consistent with a very high potential of *S. reticulata* for bioenergy purposes.

**Acknowledgments**

We thank Maria Tereza Fernandes Piedade (INPA) for all the support with seed collection and Eglee Igarashi (LAFIECO) for HPLC analyses. This work was supported by the National Institute of Science and Technology of Bioethanol (INCT-Bioethanol) (FAPESP 2008/57908-6 and 2014/50884-5; National Council for Scientific and Technological Development CNPq 574002/2008-1 and 465319/2014-9), the Ministry of Science and Technology of Brazil, Eletronorte (Pará, Brazil), and Centro de Processos Biológicos e Industriais para Biocombustíveis-CeProBIO (FAPESP 2009/52840-7 and CNPq 490022/2009-0). AG and BCAM are thankful to CNPq and MCMM thanks FAPESP (18/03764-5) for their fellowships.

**Conflicts of interest**

The authors declare that they have no conflict of interest.

**References**

1. IPCC (2014) Synthesis report. Contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. Geneva, Switzerland

2. Karp A, Richter GM (2011) Meeting the challenge of food and energy security. J Exp Bot 62:3263–3271. https://doi.org/10.1093/jxb/err099

3. de Souza AP, Grandis A, Leite DCC, Buckeridge MS (2014) Sugarcane as a bioenergy source: History, performance, and perspectives for second-generation bioethanol. BioEnergy Res 7:24–35. https://doi.org/10.1007/s12155-013-9366-8

4. Richard TL (2010) Challenges in scaling up biofuels infrastructure. Science 329:793–796. https://doi.org/10.1126/science.1189139

5. Bordonal R de O, Carvalho JLN, Lal R, et al (2018) Sustainability of sugarcane production in Brazil. A review. Agron Sustain Dev 38:13. https://doi.org/10.1007/s13593-018-0490-x

6. Milner S, Holland RA, Lovett A, et al (2016) Potential impacts on ecosystem services of land use transitions to second-generation bioenergy crops in GB. GCB Bioenergy 8:317–333. https://doi.org/10.1111/gcbb.12263

7. Pandey A, Larroche C, Dussap C-G, et al (2019) Biomass, biofuels, biochemicals, Second. Elsevier

8. Liu C-G, Xiao Y, Xia X-X, et al (2019) Cellulosic ethanol production: Progress, challenges and strategies for solutions. Biotechnol Adv 37:491–504. https://doi.org/https://doi.org/10.1016/j.biotechadv.2019.03.002

9. Ruane J, Sonnino A, Agostini A (2010) Bioenergy and the potential contribution of agricultural biotechnologies in developing countries. Biomass and Bioenergy 34:1427–1439. https://doi.org/https://doi.org/10.1016/j.biombioe.2010.04.011

10. Duarte AR, Bezerra UH, de Lima Tostes ME, et al (2010) A proposal of electrical power supply to Brazilian Amazon remote communities. Biomass and Bioenergy 34:1314–1320. https://doi.org/https://doi.org/10.1016/j.biombioe.2010.04.004

11. Cardoso D, Särkinen T, Alexander S, et al (2017) Amazon plant diversity revealed by a taxonomically verified species list. Proc Natl Acad Sci 114:10695–10700. https://doi.org/10.1073/pnas.1706756114

12. Parolin P (2005) *Senna reticulata* (Willd.) H. S. Irwin & Barneby (Fabaceae) as “Pasture Killer” (“Matapasto”) pioneer tree in amazonian floodplains. Ecol Apl 4:41–46

13. Arenque BC, Grandis A, Pocius O, et al (2014) Responses of *Senna reticulata*, a legume tree from the Amazonian floodplains, to elevated atmospheric CO2 concentration and waterlogging. Trees 28:1021–1034. https://doi.org/10.1007/s00468-014-1015-0

14. Parolin P (1999) Growth strategies of *Senna reticulata* and *Cecropia latiloba*, two pioneer tree species of Central Amazonian floodplains? Bielefelder Ökologische Beiträge 272–277

15. Parolin P, Oliveira AC, Piedade MTF, et al (2002) Pioneer trees in Amazonian floodplains: Three key species form monospecific stands in different habitats. Folia Geobot 37:225–238. https://doi.org/10.1007/BF02804233

16. Parolin P (2002) Seasonal changes of specific leaf mass and leaf size in trees of Amazonian floodplain. Phyton (B Aires) 42:169–185

17. Parolin P (2002) Submergence tolerance vs. escape from submergence: two strategies of seedling establishment in Amazonian floodplains. Environ Exp Bot 48:177–186. https://doi.org/https://doi.org/10.1016/S0098-8472(02)00036-9

18. Carpita NC, Kanabus J (1987) Extraction of starch by dimethyl sulfoxide and quantitation by enzymatic assay. Anal Biochem 161:132–139. https://doi.org/https://doi.org/10.1016/0003-2697(87)90662-2

19. Gorshkova TA, Wyatt SE, Salnikov V V, et al (1996) Cell-wall polysaccharides of developing flax plants. Plant Physiol 110:721–729. https://doi.org/10.1104/pp.110.3.721

20. Carpita NC (1984) Fractionation of hemicelluloses from maize cell walls with increasing concentrations of alkali. Phytochemistry 23:1089–1093. https://doi.org/https://doi.org/10.1016/S0031-9422(00)82615-1

21. Updegraff DM (1969) Semimicro determination of cellulose inbiological materials. Anal Biochem 32:420–424. https://doi.org/https://doi.org/10.1016/S0003-2697(69)80009-6

22. Saeman JF, Bubl JL, Harris EE (1945) Quantitative saccharification of wood and xellulose. Ind Eng Chem Anal Ed 17:35–37. https://doi.org/10.1021/i560137a008

23. Kim J-B, Carpita NC (1992) Changes in esterification of the uronic acid groups of cell wall polysaccharides during elongation of maize coleoptiles. Plant Physiol 98:646–653. https://doi.org/10.1104/pp.98.2.646

24. Filisetti-Cozzi TMCC, Carpita NC (1991) Measurement of uronic acids without interference from neutral sugars. Anal Biochem 197:157–162. https://doi.org/https://doi.org/10.1016/0003-2697(91)90372-Z

25. dos Santos WD, Ferrarese MLL, Nakamura C V, et al (2008) Soybean (*Glycine max*) root lignification induced by ferulic acid. The possible mode of action. J Chem Ecol 34:1230. https://doi.org/10.1007/s10886-008-9522-3

26. Bruce RJ, West CA (1989) Elicitation of lignin biosynthesis and isoperoxidase activity by pectic fragments in suspension cultures of castor bean. Plant Physiol 91:889–897. https://doi.org/10.1104/pp.91.3.889

27. Gomez LD, Whitehead C, Barakate A, et al (2010) Automated saccharification assay for determination of digestibility in plant materials. Biotechnol Biofuels 3:23. https://doi.org/10.1186/1754-6834-3-23

28. Carpita NC, Defernez M, Findlay K, et al (2001) Cell wall architecture of the elongating maize coleoptile. Plant Physiol 127:551-565. https://doi.org/10.1104/pp.010146

29. Cosgrove DJ (1997) Assembly and enlargements of the primary cell wall in plants. Annu Rev Cell Dev Biol 13:171–201. https://doi.org/10.1146/annurev.cellbio.13.1.171

30. Albersheim P, Darvill A, Roberts K, Sederoff R SA (2011) Plant cell walls. From chemistry to biology. Ann Bot 108:viii–ix. https://doi.org/10.1093/aob/mcr128

31. DeMartini JD, Pattathil S, Miller JS, et al (2013) Investigating plant cell wall components that affect biomass recalcitrance in poplar and switchgrass. Energy Environ Sci 6:898–909. https://doi.org/10.1039/C3EE23801F

32. Salazar MM, Grandis A, Pattathil S, et al (2016) Eucalyptus cell wall architecture: Clues for lignocellulosic biomass deconstruction. BioEnergy Res 9:969–979. https://doi.org/10.1007/s12155-016-9770-y

33. Selvendran RR (1985) Developments in the chemistry and biochemistry of pectic and hemicellulosic polymers. J Cell Sci 1985:51–88. https://doi.org/10.1242/jcs.1985.Supplement\_2.4

34. Seymour GB, Colquhoun IJ, Dupont MS, et al (1990) Composition and structural features of cell wall polysaccharides from tomato fruits. Phytochemistry 29:725–731. https://doi.org/https://doi.org/10.1016/0031-9422(90)80008-5

35. Pattathil S, Avci U, Miller JS, Hahn MG (2012) Immunological approaches to plant cell wall and biomass characterization: Glycome profiling. In: Himmel M (ed) Biomass Conversion. Methods in Molecular Biology. Humana Press, Totowa, NJ, pp 61–72

36. Atmodjo MA, Hao Z, Mohnen D (2013) Evolving views of pectin biosynthesis. Annu Rev Plant Biol 64:747–779. https://doi.org/10.1146/annurev-arplant-042811-105534

37. Scheller HV, Ulvskov P (2010) Hemicelluloses. Annu Rev Plant Biol 61:263–289. https://doi.org/10.1146/annurev-arplant-042809-112315

38. De Simone O, Haase K, Müller E, et al (2003) Apoplasmic barriers and oxygen transport properties of hypodermal cell walls in roots from four Amazonian tree species. Plant Physiol 132:206–217. https://doi.org/10.1104/pp.102.014902

39. Nishiuchi S, Yamauchi T, Takahashi H, et al (2012) Mechanisms for coping with submergence and waterlogging in rice. Rice 5:2. https://doi.org/10.1186/1939-8433-5-2

40. Moog PR, Janiesch P (1990) Root growth and morphology of carex species as influenced by oxygen deficiency. Funct Ecol 4:201–208. https://doi.org/10.2307/2389339

41. Colmer TD, Gibberd MR, Wiengweera A, Tinh TK (1998) The barrier to radial oxygen loss from roots of rice (*Oryza sativa* L.) is induced by growth in stagnant solution. J Exp Bot 49:1431–1436. https://doi.org/10.1093/jxb/49.325.1431

42. Poorter H (1993) Interspecific variation in the growth response of plants to an elevated ambient CO2 concentration. In: Rozema J, Lambers H, Van de Geijn S, Cambridge M (eds) CO2 and biosphere. Advances in vegetation science. Springer, Dordrecht, pp 77–98

43. Ainsworth EA, Rogers A (2007) The response of photosynthesis and stomatal conductance to rising [CO2]: mechanisms and environmental interactions. Plant Cell Environ 30:258–270. https://doi.org/10.1111/j.1365-3040.2007.01641.x

44. De Souza AP, Gaspar M, Da Silva EA, et al (2008) Elevated CO2 increases photosynthesis, biomass and productivity, and modifies gene expression in sugarcane. Plant Cell Environ 31:1116–1127. https://doi.org/10.1111/j.1365-3040.2008.01822.x

45. Dusenge ME, Duarte AG, Way DA (2019) Plant carbon metabolism and climate change: elevated CO2 and temperature impacts on photosynthesis, photorespiration and respiration. New Phytol 221:32–49. https://doi.org/10.1111/nph.15283

46. Poorter H, Van Berkel Y, Baxter R, et al (1997) The effect of elevated CO2 on the chemical composition and construction costs of leaves of 27 C3 species. Plant Cell Environ 20:472–482. https://doi.org/10.1046/j.1365-3040.1997.d01-84.x

47. Körner C (2015) Paradigm shift in plant growth control. Curr Opin Plant Biol 25:107–114. https://doi.org/https://doi.org/10.1016/j.pbi.2015.05.003

48. Saxe H, Ellsworth DS, Heath J (1998) Tree and forest functioning in an enriched CO2 atmosphere. New Phytol 139:395–436. https://doi.org/10.1046/j.1469-8137.1998.00221.x

49. Ezquer I, Salameh I, Colombo L, Kalaitzis P (2020) Plant cell walls tackling climate change: Insights into plant cell wall remodeling, its regulation, and biotechnological strategies to improve crop adaptations and photosynthesis in response to global warming. Plants 9:1–27. https://doi.org/10.3390/plants9020212

50. Kinsman EA, Lewis C, Davies MS, et al (1997) Elevated CO2 stimulates cells to divide in grass meristems: a differential effect in two natural populations of Dactylis glomerata. Plant Cell Environ 20:1309–1316. https://doi.org/10.1046/j.1365-3040.1997.d01-21.x

51. Armstrong W (1980) Aeration in higher plants. Adv Bot Res 7:225–332. https://doi.org/10.1016/S0065-2296(08)60089-0

52. Colmer TD (2002) Aerenchyma and an inducible barrier to radial oxygen loss facilitate root aeration in Upland, Paddy and Deep‐water Rice (*Oryza sativa* L.). Ann Bot 91:301–309. https://doi.org/10.1093/aob/mcf114

53. Gibeaut DM, Cramer GR, Seemann JR (2001) Growth, cell walls, and UDP-Glc dehydrogenase activity of *Arabidopsis thaliana* grown in elevated carbon dioxide. J Plant Physiol 158:569–576. https://doi.org/https://doi.org/10.1078/0176-1617-00229

54. Ferris R, Taylor G (1994) Increased root growth in elevated CO2: a biophysical analysis of root cell elongation. J Exp Bot 45:1603–1612. https://doi.org/10.1093/jxb/45.11.1603

55. Ranasinghe S, Taylor G (1996) Mechanism for increased leaf growth in elevated CO2. J Exp Bot 47:349–358. https://doi.org/10.1093/jxb/47.3.349

56. Taylor G, Ranasinghe S, Bosac C, et al (1994) Elevated CO2 and plant growth: cellular mechanisms and responses of whole plants. J Exp Bot 45:1761–1774. https://doi.org/10.1093/jxb/45.Special\_Issue.1761

57. Carroll A, Somerville C (2009) Cellulosic biofuels. Annu Rev Plant Biol 60:165–182. https://doi.org/10.1146/annurev.arplant.043008.092125

58. Röder M, Thornley P (2018) Waste wood as bioenergy feedstock. Climate change impacts and related emission uncertainties from waste wood based energy systems in the UK. Waste Manag 74:241–252. https://doi.org/https://doi.org/10.1016/j.wasman.2017.11.042

59. Ulloa Ulloa C, Acevedo-Rodríguez P, Beck S, et al (2017) An integrated assessment of the vascular plant species of the Americas. Science 358:1614–1617. https://doi.org/10.1126/science.aao0398

60. Karp A, Shield I (2008) Bioenergy from plants and the sustainable yield challenge. New Phytol 179:15–32. https://doi.org/10.1111/j.1469-8137.2008.02432.x

61. Yuan JS, Tiller KH, Al-Ahmad H, et al (2008) Plants to power: bioenergy to fuel the future. Trends Plant Sci 13:421–429. https://doi.org/10.1016/j.tplants.2008.06.001

62. Somerville C, Youngs H, Taylor C, et al (2010) Feedstocks for lignocellulosic biofuels. Science 329:790–792. https://doi.org/10.1126/science.1189268

63. Frederick WJ, Lien SJ, Courchene CE, et al (2008) Production of ethanol from carbohydrates from loblolly pine: A technical and economic assessment. Bioresour Technol 99:5051–5057. https://doi.org/https://doi.org/10.1016/j.biortech.2007.08.086

64. Romaní A, Garrote G, Alonso JL, Parajó JC (2010) Bioethanol production from hydrothermally pretreated Eucalyptus globulus wood. Bioresour Technol 101:8706–8712. https://doi.org/https://doi.org/10.1016/j.biortech.2010.06.093

65. Huber GW, Chheda JN, Barrett CJ, Dumesic JA (2005) Production of liquid alkanes by aqueous-phase processing of biomass-derived carbohydrates. Science 308:1446–1450. https://doi.org/10.1126/science.1111166

66. Huber GW, Iborra S, Corma A (2006) Synthesis of transportation fuels from biomass: Chemistry, catalysts, and engineering. Chem Rev 106:4044–4098. https://doi.org/10.1021/cr068360d

67. Vanholme R, Morreel K, Ralph J, Boerjan W (2008) Lignin engineering. Curr Opin Plant Biol 11:278–285. https://doi.org/https://doi.org/10.1016/j.pbi.2008.03.005

68. Poovaiah CR, Nageswara-Rao M, Soneji JR, et al (2014) Altered lignin biosynthesis using biotechnology to improve lignocellulosic biofuel feedstocks. Plant Biotechnol J 12:1163–1173. https://doi.org/10.1111/pbi.12225

69. Loqué D, Scheller H V, Pauly M (2015) Engineering of plant cell walls for enhanced biofuel production. Curr Opin Plant Biol 25:151–161. https://doi.org/https://doi.org/10.1016/j.pbi.2015.05.018

70. Kang X, Kirui A, Dickwella Widanage MC, et al (2019) Lignin-polysaccharide interactions in plant secondary cell walls revealed by solid-state NMR. Nat Commun 10:347. https://doi.org/10.1038/s41467-018-08252-0

71. Chen F, Dixon RA (2007) Lignin modification improves fermentable sugar yields for biofuel production. Nat Biotechnol 25:759–761. https://doi.org/10.1038/nbt1316

72. Bassam NE (1998) Energy plant species: Their use and impact on environment and development. James & James, London, UK

73. Mascal M, Nikitin EB (2008) Direct, high-yield conversion of cellulose into biofuel. Angew Chemie Int Ed 47:7924–7926. https://doi.org/10.1002/anie.200801594

74. Deckmyn G, Laureysens I, Garcia J, et al (2004) Poplar growth and yield in short rotation coppice: Model simulations using the process model SECRETS. Biomass and Bioenergy 26:221–227. https://doi.org/https://doi.org/10.1016/S0961-9534(03)00121-1

**Figure legends**

**Figure 1.** The Amazon tree *Senna reticulata*. **A** and **B.** Juvenile individuals; **C.** Flowers; **D.** Adult individual; **E.** *S. reticulata* in a monospecific stand. This species grows preferentially at abandoned pastures with high nutrient availability.

**Figure 2.** **A.** Lignin content (LTGA mg. g-1); Saccharification pretreatment with **B.** alkaline solution (nmol/g.biomass/hour) or **C.** hot water (nmol/g.biomass/hour); **D.** Person correlation between saccharification/alkaline and lignin content in all organs (-0.938 and *P* = 0.000) and **E.** saccharification/water and starch percentage in biomass (+0.772 and *P* = 0.000). Measurements were made in leaves, stems, and roots of *Senna reticulata* plants grown during 90 days under ambient (AMB, white bars) or elevated CO2 (ELEV, black bars) treatments. Data are mean ± SE of 5 replicates. Significant differences (Student’s *t*-test, *P* < 0.05) between treatments are indicated by asterisks.

**Table legends**

**Table 1.** Cell wall fractionation yields (%) considering total biomass or only cell wall material insoluble residue in leaves, stems, and roots of *Senna reticulata* plants grown during 90 days under Ambient (AMB) or Elevated CO2 (ELEV) treatments. Data are mean ± SE of five replicates. Significant differences (Student’s *t*-test, *P* < 0.05) between treatments are indicated in bold.

**Table 2.** Comparison of monosaccharides (%) and uronic acids (µg. mg-1) in cell wall fractions among leaves, stems, and roots of *Senna reticulata* plants grown during 90 days. Data are mean ± SE of 10 replicates. Significant differences (one-way ANOVA, followed by Tukey test, *P* < 0.05) among organs are indicated in bold.

**Table 3.** Monosaccharides (%), hexose: pentose ratios, and uronic acid (µg. mg-1) in cell wall fractions of **leaves** of Senna reticulata plants grown during 90 days under ambient (AMB) or elevated CO2 (ELEV) treatments. Data are mean ± SE of 5 replicates. Significant differences (Student’s t-test, P < 0.05) between treatments are indicated in bold.

**Table 4.** Monosaccharides (%), hexose: pentose ratios, and uronic acid (µg. mg-1) in cell wall fractions of **stems** of *Senna reticulata* plants grown during 90 days under ambient (AMB) or elevated CO2 (ELEV) treatments. Data are mean ± SE of 5 replicates. Significant differences (Student’s *t*-test, *P* < 0.05) between treatments are indicated in bold.

**Table 5.** Monosaccharides (%), hexose: pentose ratios, and uronic acid (µg. mg-1) in cell wall fractions of **roots** of *Senna reticulata* plants grown during 90 days under ambient (AMB) or elevated CO2 (ELEV) treatments. Data are mean ± SE of 5 replicates. Significant differences (Student’s *t*-test, *P* < 0.05) between treatments are indicated in bold.

***Supplemental material***

**Supplemental 1.** Percentage of cell wall fractionation yield in leaves, stem and roots in relation to biomass or cell wall content of *Senna reticulata*. Data are mean ± SE of 10 replicates. Means showing significant changes in organs (*P* < 0.05) are indicated by letters.