Afforestation With Tropical N-Fixing Species in The Brazilian Atlantic Rainforest: Carbon and Nitrogen in The Soil Profile

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Abstract

Aims

Atlantic Rainforest biome is one of the most threatened in the world by deforestation where afforestation programs are urgently needed. N-fixing species should be prioritized in re-establishing forest covers as they can enhance soil C and N and stimulate cycling of other nutrients. Yet, tropical ecosystems play a key role in global warming and remain underestimated in the global biogeochemical balances. We aimed to investigate the effects of tropical N-fixing species on soil C and N pools after pasture conversion

Methods

We selected: *Plathymenia reticulata*, *Hymenaea courbaril*, and *Centrolobium tomentosum* 27-year-old monospecific stands. We evaluated soil organic carbon (SOC), nitrogen (STN), and the natural abundance of ^{13}C and ^{15}N in the soil profile up to 100 cm depth.

Results

SOC was higher for *P. reticulata*, but an opposite pattern was observed when combining only soil layers up to 30 cm soil depth. Meanwhile, STN was similar across species and $\delta^{15}\text{N}$ values showed enrichment at intermediate soil layers indicating ^{14}N gaseous loss. Most of the SOC originated from the planted trees rather than the former pasture, except beneath *C. tomentosum* where C_4 derived C is decreasing at a slower rate.

Conclusion

This study presents novel insights in the understanding of tropical N-fixing species effects on soil C and N where specific-species traits appear to mediate SOC retention to the mineral soil rather than the N-fixing ability *per se*.

Introduction

The Atlantic Rainforest biome has high species diversity and a high level of endemism and it has been identified as one of the world's hotspots of biodiversity (Myers et al. 2000). Despite its ecological relevance, the biome is one of the most threatened by deforestation on earth where only 9-12% of the original vegetation remains existing in small, isolated, and unprotected fragments (Ribeiro et al. 2009; SOS Mata Atlântica 2020).

The main causes of the Atlantic Rainforest fragmentation are clearing for crops, timber, animal grazing, firewood, and urbanization. Given this scenario, forest plantations to recover the biome are urgently needed and, if well managed, revegetation of degraded sites can also contribute to meet market demand of timber and non-timber products, therefore, reducing illegal logging (Barroso et al. 2018).

In the context of the Atlantic Rainforest's species diversity, the experience with its species and their interaction with soil fertility is limited, and knowledge of tropical N-fixing species is even scarcer, recognized as a current gap in the literature (Mayer et al. 2020). By biological nitrogen fixation, N-fixers can associate with bacteria and convert atmospheric N_2 to ammonia (NH_3). The continuous N accretion to the soil system tends to stimulate cycling of other nutrients (Chaer et al. 2011; Mendes et al. 2021).

More specifically, N-fixing tree species have been reported to exert a key role in enhancing terrestrial carbon (C) concentration and stocks when compared to non-N-fixing species (Luo et al. 2004; Forrester et al. 2013; Mayer et al. 2020) with N inputs increasing +11.8% mineral soil C storage as reported by Nave et al. (2009) in a meta-analysis only including temperate forests. At a global scale, the higher soil C even beneath tropical N-fixing tree species was confirmed by Peng et al. (2020). The greater accumulation of fresh C plus the retention of old soil C are the main assumptions to explain the increased soil C under N-fixing tree species (Resh et al. 2002), however, the underlying processes remain elusive (Peng et al. 2020).

N-fixing tree species has been prioritized in afforestation programs as the improvements for soil fertility will be helpful for stand productivity and where more demanding trees can grow (Chaer et al. 2011; Forrester et al. 2013; Mayer et al. 2020). Shifting cultivation e.g. pasture to tropical N-fixing species can restore C and N to the levels found in the soil under native vegetation in a short period of time (Macedo et al. 2008; Chaer et al. 2011), since new C and N inputs will occur through above and belowground plant litter (Hoogmoed et al. 2014b; Vicente et al. 2016; Ngaba et al. 2020). However, C sequestration, as well as N retention and their vertical distribution in the soil, will be mediated by specific functional traits of each species such as litter productivity and litter chemical composition (Thomas and Prescott 2000; Vesterdal et al. 2008; Li et al. 2020; Peng et al. 2020).

To better understand the fate of litter C and N and their transformation and pools in the soil profile after land-use changes, stable isotopic fractionation has been applied as a powerful tool (Hobbie and Ouimette 2009; Liu et al. 2018b; Paul et al. 2019). The technique is also effective to track land-use changes' effects on soil organic matter (SOM) origin (Conen et al. 2008; Vicente et al. 2016; Carvalho et al. 2017).

Apart from the soil fertility improvement, soil C storage in the soil profile has a particular contribution for mitigating greenhouse gas fluxes acting as a great sink or source within the climate change context. Its relevance is due to large amounts (approximately 2344 Gt) of organic C stored in the soil globally (Stockmann et al. 2013) with a large contribution from forest ecosystems (Pan et al. 2011) where deforestation of tropical areas have a significant impact on the global C cycle (Silver et al. 2000; Vilella et al. 2012).

Evidence of litterfall N association with soil N status was already observed under different European common gardens (Vesterdal et al. 2008), and in accordance with the meta-analysis performed by Zheng et al. (2021), an average of 24% of the C and 58% of the N lost from litter were recovered to the soil

regardless of experimental conditions. Yet, the authors concluded that proportionally higher C and N were transferred to the soil from slowly than rapidly decomposing litters.

A previous study showed that two even-aged tropical N-fixing species returned different amounts of C and N via leaf litter where *Plathymenia reticulata* had higher C and N than *Hymenaea courbaril* with similar decay rates (Siqueira et al. *in press*). Thus, given these differences, a first question arose: Does *P. reticulata* have enhanced soil C and N than *H. courbaril*? To test that and to have a greater overview of tropical N-fixing species we also included in the study *Centrolobium tomentosum* (with similar leaf litter C and N contents to *H. courbaril*). This study aimed to answer a second question: Did tropical N-fixing species with different leaf litter C and N contents recover C and N to the soil consistently with litter contents? The quantification considers 0-30 cm soil grouped individually and the soil profile up to 100 cm.

The three species are located at the same study site that was previously a pasture where single monospecific forest stands were planted 27 years ago. Due to the historical land-use change back in time, we targeted our third question based on ^{13}C isotope labeling: How much did the pasture conversion to 27-year-old tropical N-fixing plantations change the origin of the current SOM?

Lastly, our fourth question was based on the well-established knowledge that tropical sites are not N-limited (Martinelli et al. 1999) and might have ^{15}N -enriched N in the soil mainly due to gaseous ^{14}N differential loss (Houlton et al. 2006; Hobbie and Ouimette 2009; Peri et al. 2012). Do tropical N-fixing species have similar patterns regarding ^{15}N enrichment/depletion in the soil profile?

The first objective related to the question 1 and 2 was quantification of SOC beneath N-fixing species with different leaf litter C and N contents individually to a layer of 0-30 and also up to 100 cm. The second objective related to question 3 was quantification of the contribution of C_3 -derived C and C_4 -derived C after pasture conversion to tropical N-fixing tree plantations. Finally, the third objective related to the fourth question was to understand vertical ^{15}N fluctuation among tropical N-fixing tree plantations after land-use change.

Materials And Methods

2.1 Site descriptions

The study was carried out in the mountain region of Rio de Janeiro, Brazil, within the municipal limits of Trajano de Moraes (22° 04' 32" S 42° 03' 51" W) in the Atlantic Rainforest biome domain. The average temperature is between 18 and 24 °C and annual precipitation of 1100 mm. The altitude is 700 m above sea level. The soil is a Typic Haplohumults (Soil Survey Staff, 2014) with low activity clay.

The site was previously forest followed by a pasture (no information for how long) that had experienced erosion and unplanned fires. In 1992, Atlantic Forest species were planted to recover the area. The

plantation was done as minimum tillage, with manual weeding, 0.40 x 0.40 x 0.40 m planting pits, use of cattle manure (10L per pit), and 10-28-06 NPK fertilizer (100 g per plant) at planting time.

A total of 49 seedlings of each tree species were planted in single plots and spaced at 3 x 3 m. Surrounding the plots, there are equidistant tree plantations and a natural forest fragment that could provide seeds for understory regeneration. Additionally, floristic inventory and diversity assessments did not display presence of grasses in any of the forest plots (unpublished data) indicating that the forest stands are composed mainly of C₃ vegetation.

We selected three forest species belonging to the Fabaceae family: *Plathymenia reticulata* Benth, *Hymenaea courbaril* Linnaeus, and *Centrolobium tomentosum* Guillem ex Benth. The species had survival rate of ³ 90%, average height ranging from 13 to 15 m and the diameter at breast height ca. 13 cm for *H. courbaril* and *C. tomentosum* and 24 cm for *P. reticulata* (Barroso et al. 2018). The species also have a great environmental and economic interest in timber and non-timber products (Cartaxo et al. 2010; Della Torre et al. 2011; Erbano and Duarte 2012; Calderón-Peralta et al. 2017; Gombeau et al. 2019; Siqueira et al. 2021).

Yet, a 50-year-old unfertilized pasture composed of *Brachiaria decumbens* and a Secondary Forest (upper canopy logged approximately 35 years ago) were considered as references for C₄ and C₃ vegetation covers, respectively, and used for calculations with soil ¹³C fractionation data beneath the aforementioned species. These areas are also located in the Atlantic Rainforest biome (21° 07' 50" S 42° 21' 59" W) and were evaluated by Vicente et al., (2016).

2.2 Soil sampling and analysis

Mineral soil was sampled in the central planting rows between trees (approximately 40 cm from the trunk basis) in the following layers: 0-10 cm; 10-20 cm; 20-30 cm; 30-40 cm; 40-50 cm; 50-75 cm and 75-100 cm, with four replicates per layer in each forest stand. For soil physical characterization, soil samples from 0-10 and 10-20 cm soil layers were considered individually beneath each forest stand.

However, for soil layers deeper than 20 cm, soil samples were collected from a single trench dug up to 100 cm and we considered them representative for all the forest stands given the soil similarity beneath them. Samples were air-dried and sieved (2 mm sieve) for soil particle size determination. The volumetric ring method was used to determine soil bulk density (Embrapa 1997). The soil physical characterization is described in Table 1.

Table 1. Particle size fractions and soil bulk density up to 100 cm under different tropical N-fixing tree species in the Southeast Brazil

Soil layer (cm)	Particle size fraction (%) and bulk density - BD- (g cm ⁻³)	Forest species		
		<i>C.tomentosum</i>	<i>H. courbaril</i>	<i>P. reticulata</i>
0-10	Sand	45	40	38
	Silt	23	24	22
	Clay	32	36	40
	BD	1.130	1.202	1.010
10-20	Sand	45	42	32
	Silt	14	13	10
	Clay	36	44	57
	BD	1.320	1.320	1.201
20-30	Sand	32	32	32
	Silt	10	10	10
	Clay	57	57	57
	BD	1.098	1.098	1.098
30-40	Sand	26	26	26
	Silt	9.6	9.6	9.6
	Clay	63	63	63
	BD	1.159	1.159	1.159
40-50	Sand	26	26	26
	Silt	8.2	8.2	8.2
	Clay	65	65	65
	BD	1.105	1.105	1.105
50-75	Sand	26	26	26
	Silt	6.7	6.7	6.7
	Clay	66	66	66
	BD	1.124	1.124	1.124
75-100	Sand	26	26	26

Silt	5.9	5.9	5.9
Clay	68	68	68
BD	1.121	1.121	1.121

The soil organic carbon (SOC) and total nitrogen (STN) contents were determined by dry combustion in an automated elemental analyzer (CHNS/O analyzer). Then, considering soil layer thickness, the soil bulk density at each layer was multiplied by SOC or STN to calculate SOC and STN stocks stored in the soil profile, expressed in Mg ha⁻¹. SOC was then corrected by the clay content of the soil as the organic matter fluctuations are related to soil texture (Moraes et al. 1996).

The natural abundance of ¹³C and ¹⁵N were determined using the Isotope Ratio Mass Spectrometer Delta V Advantage (IRMS - Thermo Scientific) coupled with Organic Elemental Analyzer (Thermo Scientific), and the results were expressed in (‰) relative to the Pee Dee Belemnite (PDB) and atmospheric N₂ International standards, respectively, calculated using the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}} - R_{\text{reference}}) / R_{\text{reference}}$$

where $R_{\text{sample}} = {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{15}\text{N} / {}^{14}\text{N}$ ratio of the sample and $R_{\text{reference}} = {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{15}\text{N} / {}^{14}\text{N}$ ratio of the reference samples

To distinguish the proportion of C derived from the previous pasture (C₄ vegetation) and from the current forest stands (C₃ vegetation) we used the equation proposed by Vitorello et al. (1989):

$$\%C\text{-}C_4 = [(d - d_a) - (d_p - d_a)] * 100,$$

where d = natural $\delta^{13}\text{C}$ abundance in the samples; d_a = natural $\delta^{13}\text{C}$ abundance in the soil samples without C₄ plant residue (Secondary forest from Vicente et al. (2016) used as reference); d_p = natural $\delta^{13}\text{C}$ abundance of the pasture plant material (-12.65‰).

Lastly, the following equation was used to obtain the % of the C₃ carbon:

$$\%C - C_3 = 100 - \%C\text{-}C_4$$

2.3 Data analysis

Considering the study site has no statistical design and no fill requirements for parametric analysis, our data was also submitted to descriptive analysis and comparison by Confidence Interval ($p < 0.05$) by Student's T-test (Rmisc package, Hope 2013) through R software (R Core Team 2019). The comparison was based on the overlapping of the Confidence Interval limits (or lack thereof), which allows to differ means with high statistical support (McGill et al. 1978).

Principal component analysis (PCA) was conducted using soil data (0-30 cm where the historical land-use change is more likely to be noticed) to evaluate a possible grouping pattern among species. For this analysis, data were standardized through z-scale transformation to avoid scale influence and plotted using ggbiplot package (Vu 2011).

Unreplicated tree stands with pseudo-replication within each species is a limitation of the present study, but frequent in forestry experiments, e.g. Lima et al. (2006); Vesterdal et al. (2002); Vicente et al. (2016). To compare tree species effects on soil C and N storage, an experimental site that limits influences from age, climate, and soil type has a crucial role but it is still rare (Oostra et al. 2006; Vesterdal et al. 2008). Given the uniqueness of these common gardens, they should not be neglected. Yet, differences in the topsoil properties among species located at the same study site were previously described by Barroso et al. (2018) also through confidence intervals which validates our analysis.

Results

3.1 Soil organic carbon (SOC) and nitrogen (STN) storage

The SOC in the 0-100 cm soil layer was higher for *P. reticulata* (214 Mg ha⁻¹) than *C. tomentosum* (171 Mg ha⁻¹) but not different from *H. courbaril* (202 Mg ha⁻¹). Conversely, regarding the 0-30 cm soil layer an opposite pattern was observed, but not statistically significant, with SOC showing the lowest value for *P. reticulata* with 84 Mg ha⁻¹ and highest for *H. courbaril* with 107 Mg ha⁻¹ (Fig.1). The STN in the 0-100 cm soil layer was similar among species ($p = 0.16$) with an average of 16 Mg ha⁻¹ and 44% (7 Mg ha⁻¹) of the STN budget is accumulated at the top 0-30 cm soil layer where no differences among species were found as well (Fig.1).

A similar pattern, but without statistically significant differences among species, was observed for SOC and STN at the individual depth intervals in the soil profile, with higher values in the uppermost soil layers followed by a slight decrease at intermediate layers and increases in the bottommost wider layers (Fig. 2).

3.2 Soil ¹³C and ¹⁵N abundance fluctuations

The natural abundance of the ¹³C was similar among forest stands with relative ¹³C-depleted C, not far from the Secondary Forest considered as a reference for C₃ vegetation. However, *C. tomentosum* had ¹³C-enriched C, closer to the Pasture, reference for C₄ vegetation, than the other species (Fig. 3a). The soil d¹⁵N values did not follow the same trajectories as d¹³C values with soil depth.

We observed relatively ¹⁵N-depleted N in the upper soil layers, but d¹⁵N values became more ¹⁵N-enriched with depth. This pattern did not follow until deeper layers but only at intermediate depths. (Fig. 3b). Vertical changes in d¹⁵N values were around ~2‰ under *P. reticulata*, approximately two times more than *C. tomentosum* and *H. courbaril* stands.

The $\delta^{13}\text{C}$ values showed higher contribution of C_4 vegetation under *C. tomentosum* even in the upper soil layers (0-10, 10-20, and 20-30 cm) when compared to *H. courbaril* and *P. reticulata* (Fig. 4).

Nevertheless, the accumulated C-C_3 at 0-10 cm layer was lower to the Secondary Forest (28.5 Mg ha^{-1}) when compared to the N-fixing species where the values ranged from 33.8 Mg ha^{-1} to 46.1 Mg ha^{-1} , but higher than *P. reticulata* at 10-20 cm soil layer.

The PCA (91.7% explanation of the data variability) showed a direct association between soil C and N stocks, and a negative correlation to PC1. The isotopic fractionation of ^{13}C and ^{15}N is indirectly correlated to soil C:N ratio indicated by the arrows in opposite directions (Fig. 5). The species were organized in different groups, where *C. tomentosum* is more associated with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ fluctuations and *P. reticulata* and *H. courbaril* associated together with soil C and N stocks and C:N ratio.

Discussion

4.1 Soil organic carbon (SOC) and total nitrogen (STN) storage

Soil C stock beneath a secondary forest located at the same biome (168 Mg ha^{-1} up to 1m depth) was similar to *C. tomentosum* but lower than *H. courbaril* and *P. reticulata*. Disturbances occurred approximately 30 years ago in the secondary forest which might have decreased above and below ground plant litter inputs as well as the decomposition dynamics (Vicente et al. 2016). However, given the time after pasture conversion to the N-fixing trees (27 years), we assumed that comparisons among them are worth it.

The higher SOC stock was observed under *P. reticulata* stand (up to 1m depth) but considering only the upper soil layers (0-30 cm) an opposite pattern was shown. The C contribution located deep down the soil profile is unlikely to be on account of the planted N-fixing tree species as changes on soil C are typically distinguishable in the forest floor and top mineral soil layers (Vesterdal et al. 2008; Guillaume et al. 2015; Xu et al. 2021). Due to the relatively short time after pasture conversion to the N-fixing stands, the soil C and N in the subsoil are probably derived from the forest vegetation even before the pasture. Therefore, to discuss N-fixing species effects on soil C and N we will limit the evaluated soil layer to 30 cm depth.

Comparing to a deforested area ($35.4 \text{ Mg C ha}^{-1}$ and 3.0 Mg N ha^{-1}) and a secondary forest with few signs of anthropic interference (58.3 Mg C and 5.4 Mg N) in the 0-30 cm soil layer, both located within the Atlantic Forest domain, (Macedo et al. 2008), the soil beneath the N-fixing species had higher values of SOC and STN.

It is well known that N-fixing species enhance soil C stocks when compared to non-N-fixing (Resh et al. 2002; Chaer et al. 2011; Hoogmoed et al. 2014b). Greater retention of old C and higher inputs of new soil C have been attributed to the N-fixing trees to explain this fact (Resh et al. 2002; Luo et al. 2004). Keeping this in mind, *P. reticulata* had the highest C and N contents returned via leaf litter (Siqueira et al. *in press*), the lowest retention of old C derived from the previous pasture (Fig. 4), and the lowest SOC stock in the 0-

30 cm soil layer. On the other hand, *C. tomentosum* had lower leaf litter C content, higher retention of old C derived from the previous pasture (at a depth of 0-30 cm) and higher SOC stock (similar to *H. courbaril* stand) in the same combined soil layers.

Pairing with a 35-year-old rubber plantation (non-N-fixer) located within similar edaphic and climatic conditions which had 95 Mg C ha⁻¹ in the top 30 cm (Vicente et al. 2016), the SOC was marginally higher than *P. reticulata*, similar to *C. tomentosum*, and lower than *H. courbaril*. It suggests that tropical N-fixing species depend more on other species-specific traits of each species such as openness canopy, litter productivity, chemical composition and structure as non-N-fixers can eventually reach similar SOC.

Different C and N addition to the soil was observed by two N-fixing tree species in a temperate climate (south-eastern Australia), and the authors also attributed to specific traits of each species and changes on microbial community composition (Hoogmoed et al. 2014a). Similar results were found by Wang et al. (2010) also comparing two N-fixing species at the top 5 cm soil layer. Due to the differences on C (and C recalcitrance), N, and P leaf litter across our species (Siqueira et al. *in press*), it is expected that decomposer community will differ and/or the microbial activity, as described by the aforementioned authors and it warrants further investigations.

Despite its higher leaf litter C content, another possible explanation for the lower SOC in the 0-30 cm soil layer under *P. reticulata* is that the leaf litter had a more recalcitrant litter with high lignin concentration (Siqueira et al. *in press*) which might have lowered the substrate use efficiency by microbes and therefore soil C (Marschner et al. 2008; Cotrufo et al. 2013). Apart from that, *P. reticulata* had higher growth rates (Barroso et al. 2018) and the uptake of nutrients might have reduced soil C and N at a layer of 0-30 cm as well (Hoogmoed et al. 2014a).

Differences in the mineral soil (0-30 cm) beneath six European tree species (non-N-fixers) were observed for N stock but not for C stock, attributed to litterfall N status. Moreover, C and N may also be incorporated into the mineral soil at a higher rate in some tree species due to different root distribution in the soil profile (Vesterdal et al. 2008). We observed an opposite pattern in our study site where differences were found only for C stocks when combining the same soil layers. It is expected that N-fixing tree species will have higher soil N as they often have higher N content in their biomass, litter, and root exudates (Hoogmoed et al. 2014b), however, the literature lacks information on the root architecture and nutrient concentration of our species.

Despite the large differences between N returned via litterfall, STN was similar across species. STN is most unlikely to be impacted by climatic factors compared to SOC (Xiang et al. 2021). However, substantial N losses appear to be occurring especially in the soil under *P. reticulata* with greater canopy openness and tendency for higher soil temperatures. Gaseous N losses frequently happen in N-saturated tropical systems due to soil capacity limitations (Bingham and Cotrufo 2016) as tropical weathered acid soils (Campo and Merino 2016).

4.2 Soil ¹³C and ¹⁵N abundance variations

Soil $\delta^{13}\text{C}$ values under the N-fixing stands were similar to Brazilian native forests (Dortzbach et al. 2015; Carvalho et al. 2017), Amazon rainforest (Araújo et al. 2011), and *Hevea brasiliensis* stand at 35 years which was previously a pasture as well (Vicente et al. 2016). However, the soil beneath *C. tomentosum* had enriched $\delta^{13}\text{C}$ values (about 1.6‰) in the uppermost ~40 cm of the soil profile explained by the mixing of fresh and old litter inputs.

It is frequently reported a relatively rapid shift from C_4 -derived C to C_3 -derived C in tropical regions mainly due to weather conditions where the warm and humid environment accelerates the organic matter decomposition rates mostly in the upper soil layers (Villela et al. 2012; Maggiotto et al. 2014). The soil C_4 -derived C losses are decreasing at a slower rate under *C. tomentosum* than *P. reticulata* and *H. courbaril* in the topsoil layers even though the temperature and humidity were the same. It is supported by the PCA where the ^{13}C soil and ^{15}N soil arrows were more related to *C. tomentosum*.

Indirectly related to climatic factors, larger canopy densities will have higher rainfall and light interception and hence low soil moisture content and soil temperatures, respectively (Liu et al. 2018; Xiang et al. 2021). The higher canopy openness in *P. reticulata* stand (Fig. 6) could also justify the lower C_4 -derived C due to rapid decomposition rates. However, *C. tomentosum* had the second higher canopy openness, but the slower decomposition rates of the old C.

When N-fixers accumulated relatively more soil N, the soil retains more C_4 -derived C (Resh et al. 2002) as higher N under N-fixing likely shift microbial community towards bacterial dominance and therefore slower organic matter decomposition rates are expected (Hoogmoed et al. 2014a). Indeed, we found a positive correlation between SOC and STN in the 0-30 cm soil layer across species but the trend is less clear when partitioning C_4 -derived C and C_3 -derived C retention.

Concerning ^{15}N fractionation, the fresh litter is relatively labile compared to organic matter in deeper soil layers and it explains ^{15}N -depleted N across species in the soil surface (Hobbie and Ouimette 2009; Ngaba et al. 2019). Likewise, the lower $\delta^{15}\text{N}$ values can also be explained by the N fixation rates as fixed N from the atmosphere has values close to zero (Martinelli et al. 1999).

The ^{15}N enrichment at intermediate depths is a frequent pattern when there is N losses of ^{14}N by gaseous nitrification and denitrification, which is common in tropical ecosystems (Houlton et al. 2006; Hobbie and Ouimette 2009; Peri et al. 2012; Nel et al. 2018). Generally, stands with an open N cycle (large pools and fluxes) seem to cause soil ^{15}N enrichment (Callesen et al. 2013). Yet, nitrification in soils could reduce soil pH by producing protons in its chemical processes (Wang et al. 2010) and the lowest pH was observed for *P. reticulata* (Barroso et al. 2018), and both facts support our previous assumption.

There is high evidence that species-specific traits such as litter chemical composition and hence microbial community are driving the SOC and STN budgets and organic matter decomposition rates more than weather conditions *per se*. Furthermore, the productivity and chemical composition of the litter from understory vegetation might have influenced C and N cycling beneath the N-fixing plantations but any

conclusion requires further investigations. Yet, a greater overview of tropical N-fixing tree species effects on SOC, STN, and isotopic fractionation has to include more species and then be investigated more closely.

Conclusion

Afforestation with tropical 27-year-old N-fixing tree plantations was able to restore soil C and N to similar or even higher rates as found in forest sites after less hostile disturbances. However, the soil beneath *P. reticulata* had enhanced soil C stored up to 100 cm depth with no differences for soil N across species.

Despite larger variations in leaf litter C and N contents among species, slight differences were observed for SOC and STN in the topsoil layers. Nevertheless, *C. tomentosum* had a slower decomposition rate of old soil C (higher C_4 - derived C left). It seems that tropical N-fixing tree species differ in C sequestration rates within mineral soil.

A similar vertical STN and ^{15}N distribution in the soil profile was observed across species with a slight tendency towards ^{15}N enrichment in the soil beneath *P. reticulata* and *C. tomentosum* stands.

Litter quality and productivity seem to be shaping the microbial community and activity differently beneath each species and hence SOC and STN pools, but any conclusion requires further investigations for a greater overview of the role N-fixing tree species on soil C and N.

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Figures

Figure 1

Soil organic carbon (SOC) and total nitrogen (STN) stocks in 0-100 cm soil layer beneath N-fixing plantations in the Southeast Brazil; Vertical bars represent $\pm$ standard deviation. Means followed by different lowercase letters differed statistically by Confidence Interval ($p < 0.05$). *ns – not significantly different ($p > 0.05$).

Figure 2

Soil organic carbon (SOC) and total nitrogen (STN) stocks at the individual layers beneath N-fixing plantations in the Southeast Brazil; Vertical bars represent $\pm$ standard deviation. *ns – means not significantly different (p>0.05).

Figure 3

Changes in $\delta^{13}\text{C}$ values (a) and $\delta^{15}\text{N}$ values (b) in the soil profile beneath N-fixing plantations in the Southeast Brazil. *Secondary forest and pasture $\delta^{13}\text{C}$ data are from Vicente et al. (2016).

Figure 4

Soil organic carbon divided into C3 plants (tree)-derived and C4 plants (pasture)-derived in the 0-30 cm soil layer beneath different tropical N-fixing tree plantations and the Secondary Forest (SF) from Vicente et al., (2016); PR – *Plathymenia reticulata*; HC – *Hymenaea courbaril*; CT – *Centrolobium tomentosum*.

Figure 5

Principal component analysis of soil variables comprising combined 0-30 cm soil layers. The area of the ellipses was determined according to the normal distribution. ^{15}N – natural abundance of $\delta^{15}\text{N}$ values in the soil; ^{13}C – natural abundance of $\delta^{13}\text{C}$ values in the soil; C stock – Soil organic carbon stock; N stock – Soil total nitrogen stock; C:N ratio – Carbon: nitrogen ratio.

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