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Indications of enhanced soil ecosystem functions in polyculture reforested grassland

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ABSTRACT

A lot of work has been done to evaluate the effects of biodiversity on ecological functions in polyculture plantings. But attention is rarely given to soil ecosystem functions such as carbon (C) sequestration and nitrogen (N) cycling even though they play a critical role in climate regulation through the sequestration and release of greenhouse gases (GHGs). In this study, stable isotopes of C and N were used to investigate if the aforementioned soil ecosystem functions are enhanced under polyculture reforestation by determining the sources of C stored in soil, its rate of incorporation, and the degree of soil N cycling. Twenty-five years after its establishment on an *Imperata cylindrica* grassland, the tree plantation has contributed 54% of the measured soil organic carbon (SOC) stock at an estimated rate of 2.41Mg C ha^{-1} yr^{-1}. Larger mean soil ^{15}N values and a more negative ^{15}N enrichment factor (ε =-5.82‰) for the whole 50cm soil depth in tree plantation indicated a better N cycling compared to grassland vegetation. Results show the potential of polyculture reforestation as a sustainable approach to restoring degraded lands and enhancing the role of soil in climate regulation by improving the capacity of soil to store C and to supply N. In addition, information generated from studies like this allow the refinement of mechanisms used in payments for ecosystem services to add more value and provide higher incentives from the ecosystem services provided by polyculture reforestation.

Keywords: stable isotopes, polyculture reforestation, payments for ecosystem services, SOC sequestration, soil nitrogen cycling

INTRODUCTION

How some ecosystem functions such as the soil carbon (C) sequestration and nitrogen (N) cycling respond to reforestation are of interest because of the role they

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play in regulating the sequestration and release of greenhouse gases (GHGs) (eg, Baldos et al 2015). However, there is limited information on the ecosystem services produced by reforestation in the tropics (De Groot & Van der Meer 2010) despite it providing a wide range of opportunities for schemes like payments for environmental services (PES). The type of approach used in any reforestation activity has long-term consequences with compromises between the structure and function of the forest. For example, the number of trees and the types of tree species planted (exotic vs native, polyculture vs monoculture plantings) and whether shrubs are included are key decisions. In a number of studies in the Philippines, the use of a combination of native tree species (polyculture planting) to reforest degraded landscapes has been found to show potential in mitigating climate change through carbon sequestration (Dierick & Hoelscher 2009), restoring ecosystem structure (Sales-Come & Hölscher 2010), and improving soil quality (Asio & Milan 2002). Polyculture tree plantations have also been observed to increase N mineralization, indicating its potential as a sustainable approach to restoring degraded lands (Mo et al 2016) and may alleviate N losses associated with nitrification and denitrification and favor N retention (Reverchon et al 2015).

One technique of particular importance in studying soil C and N dynamics is the use of stable isotope techniques. The ratio between stable isotopes (for C, $^{13}C/^{12}C$; for N, $^{15}N/^{14}N$) can be used as a marker of matter and energy flow and as an integrator to evaluate the direction and rate of ecological processes related to isotope fractionation. Understanding the natural variability in the stable C isotope composition of vegetation and soils allows us to delve deeper into the dynamics of soil C sequestration. For example, when vegetation communities change from one photosynthetic pathway to another (eg, land-use change via reforestation where tree plantations replace grasslands), stable isotope techniques allow us to estimate how much C trees actually contribute. Meanwhile, the natural abundance of N stable isotope ratio in soils can serve as an integrator of terrestrial N cycling (Robinson 2001) similar to studies done to measure soil N cycling rates in the Ecuadorian Andes (eg, Baldos et al 2015, Arnold et al 2009) and in Panama (eg, Corre et al 2010). It has the potential to characterize the N supplying capacity of a soil under a particular vegetation community, to describe spatial and temporal patterns of N cycling, as well as how disturbances alter the N cycle (eg, in Ecuador, Baldos et al 2015, & Arnold et al 2009, in Panama Corre et al 2010).

This study aimed to assess the response of soil C sequestration and N cycling to polyculture reforestation strategy by determining the sources and proportion of the soil C stock, and the qualitative description of the degree of soil N cycling through the natural abundance signatures of ^{15}N and its enrichment in the soil profile.

MATERIALS AND METHODS

Site Description

The study was conducted in VSU, Baybay City, Leyte (10°44'43.4724"N, 124°48'11.6316" E) on a polyculture tree plantation and a grassland area (Figure 1).

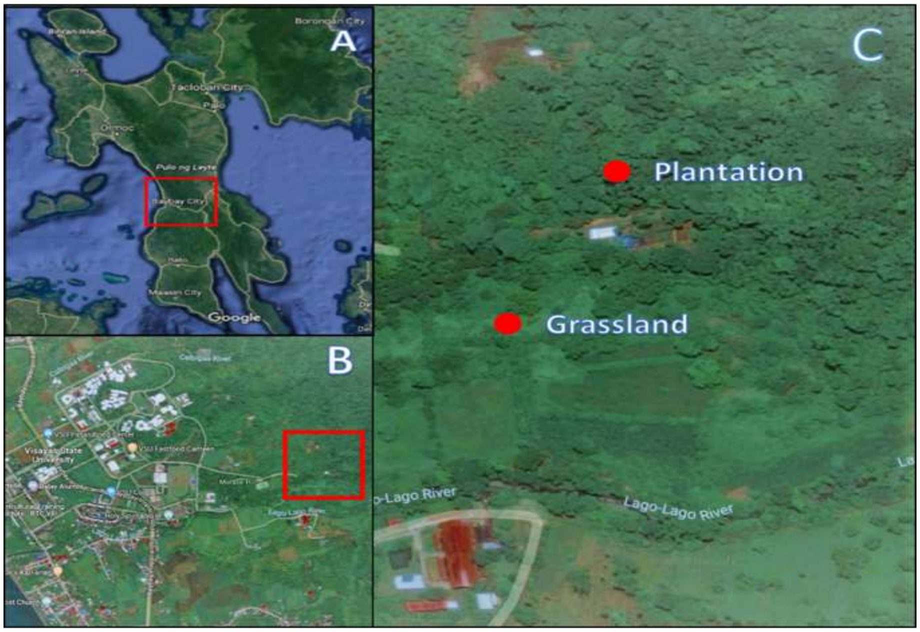

Figure 1 Location the study site in Baybay City,Leyte (A) inside the main campus of Visayas State University (B) on a polyculture tree plantation and a grassland area (C). © Google Maps 2017

The study site has been described by Navarette and Tsutsuki (2008) to be approximately 107m above sea level and soil is Typic Hapludult (Soil Taxonomy) or Haplic Alisol (Food & Agriculture Organization System) derived from andesitic pyroclastic materials of late Quaternary (probably Holocene to upper Pleistocene) origin (Asio 1996).

The polyculture tree plantation was established in 1992 on an approximately 2 hectares of former *Imperata cylindrica* grassland. It is composed of the following all C3 tree species: balobo (*Diplodiscus paniculatus*), kalumpit (*Terminalia microcarpa*), bagtikan (*Parashorea malaanonan*), hagakhak (*Dipterocapus validus*), white lauan (*Shorea contorta*), bitanghol (*Calophyllum blancoi*), mahogany (*Swietenia macrophylla*), almon (*Shorea almon*), agoho (*Casuarina equisetifolia*), yakal saplungan (*Hopea plagata*), yakal kaliot (*Hopea malibato*), almaciga (*Agathis philipinensis*), yemane (*Gmelina arborea*), and toog (*Petersianthus quadrialatus*) in a 2×4m spacing. The plantation has an average stem diameter of 16cm, and an average height of 13m. It was primarily established using the Rainforestation approach (Asio & Milan 2002) with the objective of ecological restoration. No silvicultural treatments were applied to the plantation.

The grassland site, approximately 2 hectares in size, is adjacent to the plantation site. Grass species present in the area is predominantly cogon (*Imperata cylindrica*) and carabao grass (*Paspalum conjugatum*). In addition, napier (*Pennisetum purpureum*) is grown and harvested for feeding buffaloes and horses. This grassland area is also used as grazing area. All the aforementioned grass species are C4 species. Management includes planting of napier and its harvest, and grazing of ruminants such as goats. The climate in Baybay City, Leyte is characterized as having no dry season with a very pronounced maximum rain period from November to January. The average annual temperature is 27.0°C, and the average annual rainfall rate is 2830mm yr^{-1}.

Sampling Design

The paired plots design was used in which the polyculture tree plantation was paired with and an adjacent grassland. In each land-use type, three 20×20m plots were established for sampling. Each plot was subdivided into sixteen 5×5m subplots, and three subplots were randomly selected out of the 16 to be sampled.

Leaf Collection

Using a slingshot, sample leaves from all trees inside each of the three 20×20m replicate plots were taken. In the grassland site, a 0.30×0.30m quadrat was placed at the center of each plot, wherein grass leaves were taken. Leaves were pooled together (for each plot), oven-dried at 65°C until constant weight was achieved, were ground to powder, and sieved.

Soil Data Collection

For each replicate sampling point at each depth interval (0-10cm, 10-30cm, 30-50cm), three metal soil cores (2.9cm diameter & 5.4cm height) were randomly inserted into undisturbed portions of the specified depth interval in the soil profile. The soil inside each metal core was carefully extracted, weighed, and wrapped in a labeled tin foil. Samples were brought to the Visayas State University (VSU) - College of Forestry and Environmental Science (CFES) dry laboratory for oven-drying at 105°C until constant weight of the samples was achieved. Bulk density was determined using the soil core method wherein oven-dry weight of the soil sample was divided by the inner volume of the metal soil core (2.65cm diameter & 5cm height) (Black 1965). Bulk density was used to convert C and N concentrations to unit weight per area (Mg ha^{-1}). Soil pH was determined by using a soil to solution suspension of 1:2.5. (Black 1965). Soil moisture content was determined by gravimetric method with oven - drying (Black 1965).

Stable Isotope Analysis

Analyses of stable C and N isotopes including organic carbon and total nitrogen of leaf, litter, and soil samples were performed at the Philippine Nuclear Research Institute using an automatic carbon and nitrogen analyzer (Sercon GSL flash combustion elemental analyzer, Sercon Limited, UK) coupled to an isotope ratio mass spectrometer (Sercon 20-22 continuous flow isotope ratio mass spectrometer, Sercon Limited, UK). Variations in the $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios are reported as $\delta^{13}C$ and $\delta^{15}N$ values compared against the standards, Vienna Pee Dee Belemnite and atmospheric N_2, respectively, and expressed in delta (δ) units, which represent parts per thousand (‰) deviation of the ratios from the standards (Equation 1):

$$\text{Equation}\quad 1\ \delta(‰) = \left(\frac{R_{sample}}{R_{sample}} - 1\right) \times 1000$$

where R_{sample} and $R_{standard}$ are C and N isotope rations of samples and standards respectively. Analytical precision is ±0.2‰.

Determination of Sources of Soil Organic Carbon (SOC) and Rate of Sequestration

To determine the proportion of the current SOC stock contributed by the original grassland vegetation (C4 plants) and the current polyculture tree plantation (C3 plants), two end-member mixing models were used (Equation 2 & Equation 3). The percentage of the SOC derived from C4 plants (% SOC_4) and from C3 plants (% SOC_3) in each layer was calculated according to the following equations (Balesdent & Marriotti 1996 as cited in Powers & Veldkamp 2005):

Equation 2 $$\%SOC_3 = \left(\frac{\delta^{13}C_{soil\ \mathrm{RF}} - \delta^{13}C_{soil\ \mathrm{GR}}}{\delta^{13}C_{leaf\ \mathrm{RF}} - \delta^{13}C_{leaf\ \mathrm{GR}}} \right) \times 100$$

Equation 3 $$\%SOC_4 = 100 - \%SOC_3$$

where $\delta^{13}C_{soil\ \mathrm{RF}}$ is polyculture tree plantation soil, $\delta^{13}C_{soil\ \mathrm{GR}}$ is grassland soil, $\delta^{13}C_{leaf\ \mathrm{RF}}$ is the input C signature from tree leaves, and $\delta^{13}C_{leaf\ \mathrm{GR}}$ is the input C signature from grass leaves. SOC stock in the polyculture tree plantation was multiplied by % SOC_3 and % SOC_4 to obtain SOC derived from C3 and C4 vegetation.

Determination of Degree of the Soil N Cycle

To describe the degree of the soil N cycle for the entire 50cm depth of the soil, the Rayleigh equation (Marriotti et al 1981) was used (Equation 4).

Equation 4 $$\varepsilon = (\delta_s - \delta_{so}) / \ln f$$

where ε is the ^{15}N enrichment factor, δ_s is the δ^{15}N value at various depths in the soil profile, δ_{so} is the δ^{15}N value of the input substrate (or the reference depth, which we took as the top 10cm depth) and f is the remaining fraction of the total N (ie, total N at certain depth divided by the total N at the top 10cm depth).

Data Analysis

Each of the soil characteristics (SOC, δ^{13}C, & δ^{15}N) from each of the soil metal cores for every depth were averaged. The values for every soil depth interval of each land use is reported as the mean (± standard error) of the three replicate sampling points. Differences between each land use for each depth of the parameters measured were assessed using a paired *T*-test. Levels of significance were defined at $p<0.05$. Statistical analyses were conducted using R (R Core Team 2013).

RESULTS AND DISCUSSION

Soil Physico-Chemical Properties

The polyculture tree plantation showed significant differences ($p<0.05$) in soil physico-chemical properties with the grassland site such as higher % moisture content (% MC), lower bulk density, and lower pH (Table 1). This may be attributed to litter and the roots of trees which might have enhanced infiltration and percolation of water from the surface down to the deeper layers (Binkley & Giardina 1998). There is also minimal disturbance in the plantation as opposed to the grassland which may have protected the soil structure from being destroyed. In all depths measured the polyculture tree plantation showed significantly lower pH than the grassland (Table 1). Most forest soils are strongly acid (Landon 1991) as a result of the release of organic acids during decomposition of litter layer and the subsequent leaching of bases from the surface mineral soil (Pritchett 1979). As a consequence, the species growing on the soil are likely to have a marked influence on soil acidity because of inherent differences in the base content of their litter (Binkley & Giardina 1998).

SOC stock, total N, and C:N ratios were not significantly different (p>0.05) between these two land-uses, especially in the top depths (Table 1) even though the values for the tree plantation were higher. This may have been due to the management practices in the grassland such as grazing which most likely leaves excrement behind (Smoliak et al 1972, Scheile et al 2018), making it an additional source of soil C and N thus masking differences in the values of these parameters in the top depths but not in the lower depth (Table 1). The significant differences observed for total N and C:N ratios in the lower depth (Table 1) may now have been due to the land use effect indicating that the conversion of grassland to tree plantation may have contributed to the improvement of these two parameters which also corroborates the initial findings of Asio and Milan (2002) that this type of planting improves soil quality.

Sources of SOC and Rate of Sequestration

In all depths (0-10cm, 10-30cm, 30-50cm) and for the whole soil profile (0-50cm), mean $\delta^{13}C$ values were significantly more depleted (p<0.05) under polyculture tree plantation (-25.72, -25.01, -24.93, -25.22‰, respectively) compared to that of the grassland (-16.90, -15.78, -15.19, -15.96‰, respectively). The set of $\delta^{13}C$ values in the polyculture tree plantation is typical of soils under C3 vegetation (trees; von Fischer & Tieszen 1995) while the latter values are characteristic of C4 vegetation (grasses; Santruckova et al 2000). The distinct ^{13}C signatures between the polyculture tree plantation and grassland is due to the different photosynthetic pathways used by each vegetation type. In the trees wherein the C3 pathway is used, the ribulose-bisphosphate carboxylase preferentially activates the lighter and more mobile $^{12}CO_2$ (Tiunov 2007). Accordingly, C fixed by C3 plants is poor in heavy C, thus making plant parts like leaves depleted in ^{13}C (more negative $\delta^{13}C$ values). In the C4 photosynthetic pathway used by the grasses, the absorption capacity of phosphoenolpyruvate carboxylase to activate CO_2 is very high, which makes the discrimination of heavy C much less pronounced, hence less depletion (less negative $\delta^{13}C$ values) of the heavier C isotope in the plant parts. The decomposition of plant material in the soil then leaves isotopic signatures that are distinctive of the type of photosynthetic pathway and hence vegetation type from which the source/s (C3 or C4) of the soil carbon stock may be inferred.

Table 1. Mean (± SE, n=3) of selected soil physico-chemical characteristics for every soil depth interval of polyculture and adjacent grassland in VisCA, Baybay City, Leyte. Different capital letters indicate significant differences among land uses for each soil depth at $p \leq 0.05$

Land-use	Soil Physico - Chemical Characteristics					
	Moisture Content (%)	Bulk Density (g cm^{-3})	pH (1:2.5)	SOC (Mg C ha^{-1})	Total N (Mg N ha^{-1})	C:N
0-10cm						
Polyculture	31.19 ± 4.46^{A}	1.27 ± 0.02^{A}	5.37 ± 0.31^{A}	23.02 ± 1.26^{A}	2.47 ± 0.29^{A}	9.36 ± 0.85^{A}
Grassland	12.19 ± 6.65^{B}	1.44 ± 0.04^{B}	5.96 ± 0.25^{B}	27.53 ± 3.19^{A}	2.34 ± 0.11^{A}	11.78 ± 1.91^{A}
10-30cm						
Polyculture	28.16 ± 2.02^{A}	1.31 ± 0.01^{A}	5.08 ± 0.13^{A}	43.28 ± 1.01^{A}	4.33 ± 0.29^{A}	10.03 ± 0.66^{A}
Grassland	12.08 ± 6.33^{B}	1.49 ± 0.06^{B}	6.21 ± 0.7^{B}	35.70 ± 9.94^{A}	3.04 ± 0.89^{B}	11.80 ± 1.04^{B}
30-50cm						
Polyculture	22.13 ± 3.20^{A}	1.44 ± 0.02^{A}	5.21 ± 0.28^{A}	45.18 ± 10.77^{A}	4.16 ± 0.86^{A}	10.86 ± 1.04^{A}
Grassland	9.82 ± 4.87^{B}	1.53 ± 0.03^{B}	6.43 ± 0.37^{B}	28.93 ± 10.31^{B}	2.24 ± 1.00^{B}	12.91 ± 0.64^{B}
0-50cm						
Polyculture	27.16 ± 2.73^{A}	1.34 ± 0.01^{A}	5.22 ± 0.08^{A}	111.47 ± 10.00^{A}	10.96 ± 0.82^{A}	10.17 ± 0.36^{A}
Grassland	11.36 ± 5.66^{B}	1.49 ± 0.03^{B}	6.22 ± 0.21^{B}	82.14 ± 16.73^{A}	7.11 ± 1.15^{B}	11.52 ± 1.16^{B}

Indications of enhanced soil ecosystem functions

The conversion of the grassland (C4 plants) and replacing it with a tree plantation (C3 plants), can be considered an in situ labeling of newly incorporated organic matter into the soil (Solomon et al 2002) as the relative proportions of C3 and C4 plants change following land cover change. Thus, in this study, the relative proportions of the contribution to the SOC of the original grassland following land cover change to polyculture tree plantation can be easily estimated.

The proportion of C contributed by the polyculture tree plantation (C3 vegetation) to the measured stock was ~54% after 25 years of establishment (Figure 2).

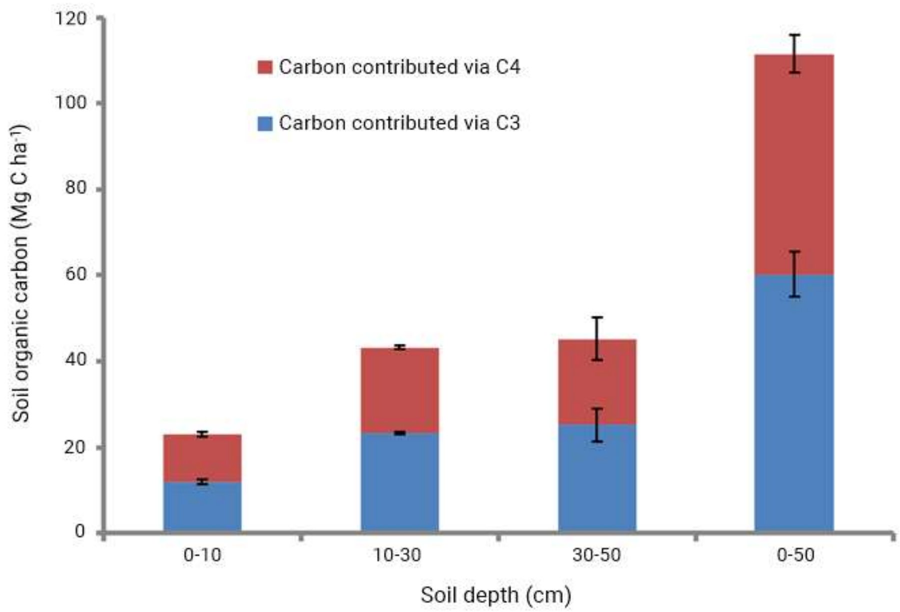

Figure 2. Mean soil organic carbon stock in the 0–50cm soil profile of the polyculture tree plantation partitioned into carbon sources (C3 vs C4)

This is comparable to the proportion of contribution of approximately 20 year-old monoculture tree plantations (*Cupressus lusitanica* & *Pinus patula*) after cultivation with maize and sorghum (C4 crops) in Ethiopia (Lemma et al 2006). Conversely, C3 isotopic signals derived from forest soil were almost totally replaced in the more labile organic matter pools by a C4 signal following 23 years of maize cultivation (Balesdent et al 1988).

Results of the study indicate that soil C deposited from the previous grassland (C4-C) may have decayed out of the SOC pool and replaced by C derived from the new polyculture tree plantation (C3-C). This loss of C4-C and its gradual replacement with C3-C may have been due to the subtle decomposition of C4-C in the SOC pool through time. The decomposition might have been aggravated by the exposure of the soil surface to more direct sunlight during the tree plantation establishment. Studies have shown that changes in land use and management practices can easily alter the SOC fraction in the soil (Six et al 2001, Lee et al 2009) and can accelerate the decomposition and mineralization of SOC (Bonde et al 1992, Wick & Tiessen 2008, Durigan et al 2016). Moreover, human activities such as vegetation clearing prior to land-use change can promote soil erosion which can disturb the carbon-rich topsoils and preferentially remove SOC (Berhe et al 2007) leading to significant declines in SOC and N stocks (Durigan et al 2016).

SOC accumulates when microbial decomposition occurs at rates lower than additions of detritus materials. The establishment of polyculture limits the addition of organic material from the original grassland vegetation. Thus, the planted trees gradually provided the organic matter input in the form of litter and this shift is evident in the distinct $\delta^{13}C$ signature of the replacement community.

Assuming a linear relationship between age of plantation and incorporation of carbon contributed by the plantation (eg, Lemma et al 2006, Powers & Veldkamp 2005) (SOC contributed by C3 or polyculture tree plantation divided by the age of plantation), this polyculture tree plantation contributes about 2.41Mg C ha^{-1} yr^{-1} of carbon to the soil. Limited studies show a wide variation in the rate of carbon sequestration in soil, depending on, among others, species used and age. In Ethiopia, Lemma et al (2006) found 3.5Mg ha^{-1} yr^{-1} of soil C sequestration rate under *C. lusitanica* and 1.5Mg ha^{-1} yr^{-1} under *P. patula*. Similarly, Zou & Bashkin (1997) found a 2.8Mg ha^{-1} yr^{-1} SOC increase in a Hawaiian eucalyptus plantation growing on an abandoned sugar cane farm for 10 years. Garten (2002) estimated an accumulation rate of 0.4 to 1.7Mg ha^{-1} yr^{-1} in the first decade following plantation of farmland. Lemenih et al (2005) found a 1.6Mg ha^{-1} yr^{-1} increase under *C. lusitanica* following 15 years of reforestation of farmland, while Bouwman & Leemans (1995) estimated a SOC accretion rate of 1.7Mg ha^{-1} yr^{-1}.

Many studies have suggested that plant communities with high species diversity may promote more efficient use of resources compared with those of less species diversity, thus leading to greater net primary production, and consequently higher C sequestration (Saha et al 2009, Meier & Bowman 2010, Wang et al 2011). In addition, ecosystems with multiple species provide some insurance that they may be steadier and continue to perform a particular function even if one of the species is lost. However, quantitative estimates of effects of tree species composition on SOC stocks under natural forest ecosystems remain scarce (Chapin III et al 2000, Berger et al 2002, Díaz-Pinés et al 2011).

Soil Nitrogen Cycle

In the three depths measured (0-10cm, 10-30cm, 30-50cm), $\delta^{15}N$ values were significantly larger (p<0.05) in the polyculture tree plantation than in the grassland (Figure 3) and were comparable to the values reported for tropical soils (eg, Baldos et al 2015, Wolf et al 2011, Lemma et al 2006, Purbopuspito et al 2006). ^{15}N enrichment factors were also larger under the plantation versus the grassland (Figure 3).

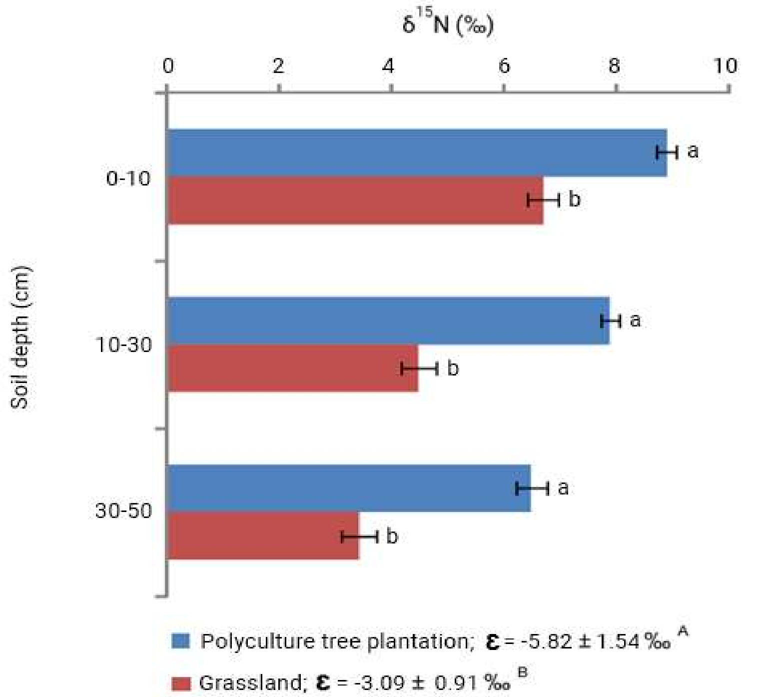

Figure 3. $\delta^{15}N$ profiles (‰; means ± SE, n=3) and ^{15}N enrichment factors (; means ± SE, n=3) of the polyculture tree plantation and grassland. Means of $\delta^{15}N$ with different small letters (per depth) and means of with different capital letters indicate significant differences (Paired T-test, $p \leq 0.05$)

Large ^{15}N natural abundance signatures and ^{15}N enrichment factors of the soil indicate high soil N cycling rates because fractionation during nitrification leaves the isotopically heavy N behind allows the loss of the isotopically light N from the ecosystem (Amundson et al 2003). These large ^{15}N values in the plantation corroborate the significantly higher total N stocks observed (Table 1). High soil N cycling rates also translate to a higher potential to supply N to plants. This could support higher net primary productivity and in turn higher input of litter to the soil leading to improved soil C sequestration.

CONCLUSION AND RECOMMENDATION

Results show the potential of polyculture reforestation as a sustainable approach to restoring degraded lands and enhancing the role of soil in climate regulation by improving the capacity of soil to store C and supply N. In addition, information generated from studies like this allow the refinement of mechanisms used in payments for ecosystem services to add more value and provide higher incentives from the ecosystem services provided by polyculture reforestation. Based on the findings, it is recommended to consider a reforestation strategy using tree species planted in mixtures, especially in degraded areas to its improve soil conditions, increase productivity, and enhance soil C sequestration and N cycling.

More studies should also be conducted to investigate the effects of tree species and its combinations on soil C sequestration and N cycling. In the future, information generated from such studies could be used in fine-tuning mechanisms for payments for ecosystem services.

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