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Leucaena leucocephala introduction into a tropical pasture in the Chaco region of Argentina. Effects on soil carbon and total nitrogen

Introducción de Leucaena leucocephala *en una pastura tropical en el Chaco argentino. Efectos en el carbono y nitrógeno total del suelo*

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Abstract

The introduction of leucaena (*Leucaena leucocephala*), apart from increasing animal production, improves soil fertility through biological nitrogen (N) fixation and its deep-rooted system. There is limited information on carbon and N dynamics in hedgerow silvopastoral systems, particularly in the subsoil profile. The concentrations and vertical distribution of organic carbon (OC) and total N , and their fractions (particulate and associate forms) in the profile (0–100 cm) of a 4-year-old leucaena stand in a *Urochloa brizantha*-*Chloris gayana* pasture were compared with those in the adjacent pure tropical grass (*U. brizantha*) pasture. Leucaena introduction increased the OC concentration in the subsoil (20–100 cm) by 45%, particularly the stable form (associate OC) in the deepest horizon (50–100 cm). This was attributed to a greater abundance of leucaena roots deeper in the profile than for grass. Leucaena also enhanced by 7.6% the N concentration (from 0.131 to 0.141%) in the topsoil (0–20 cm) associated with an increment in the labile form (particulate organic N), due to leaf deposition, recycling of animal feces and nodule-N turnover from N fixation. Leucaena establishment has the potential to improve soil fertility and hence availability of N to companion grass growth, and can be utilized as a greenhouse gas mitigation strategy.

Keywords: C sequestration, leguminous trees, soil carbon fractions, tropical grasses.

Resumen

La introducción de leucaena (*Leucaena leucocephala*), además de incrementar la producción animal, aumenta la fertilidad del suelo por fijación simbiótica de nitrógeno (N) y por sus raíces profundas. Existe poca información sobre la dinámica de carbono y N en sistemas silvopastoriles, particularmente en el subsuelo. La cantidad y distribución vertical de carbono orgánico (CO) y N total, y sus fracciones en el perfil del suelo (0–100 cm) de una pastura de leucaena de 4 años de edad en asociación con *Urochloa brizantha* y *Chloris gayana*, fueron comparadas con una pastura adyacente de *U. brizantha* en monocultivo. Leucaena incrementó en un 45% la concentración de CO (0.98 a 1.42%) en el subsuelo (20–100 cm), particularmente la forma estable (CO asociado) en el horizonte más profundo (50–100 cm), efecto atribuido a sus raíces profundas. Leucaena también acrecentó en un 7.6% la concentración de N (de 0.131 a 0.141%) en el horizonte superficial del suelo (0–20 cm), asociado al incremento de la forma lábil (N orgánico particulado), atribuido a deposición de hojas, reciclado de excreta animal y descomposición de nódulos. La implantación de leucaena tiene el potencial de mejorar la fertilidad del suelo, la disponibilidad de N para gramíneas asociadas, y puede ser una estrategia de mitigación de gases de efecto invernadero.

Palabras clave: Secuestro de carbono, árboles leguminosos, fracción de carbono, pastos tropicales.

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Introduction

Sustaining or enhancing soil organic carbon (OC) and total nitrogen (TN) in grazing systems is essential for maintaining the chemical, biological and physical properties of soils, as well as mitigating greenhouse gases emitted by agriculture (Franzluebbers and Stuedemann 2009). Tropical grass pastures are typically constrained in their capacity to store soil C due to limited plant-available N in soils under pure grass pastures and frequent overgrazing, which leads to low primary biomass production and OC losses (Dalal and Carter 2000). Legume introduction in mixtures with grasses increases soil OC and TN in grazing systems (Fisher et al. 1994; Peoples et al. 2001; Tarré et al. 2001). Planting the multipurpose forage tree legume, leucaena (*Leucaena leucocephala* ssp. *glabrata*), has been reported to improve topsoil fertility in hedgerows in silvopastoral systems (Radrizzani et al. 2011; Conrad et al. 2017) and to increase livestock productivity (Radrizzani and Nasca 2014). Although there is some information in the Chaco region on carbon sequestration in tropical grass pastures (Banegas 2014) and in silvopastoral systems (Corbella et al. 2015), there is no published information on changes in soil OC and TN levels and their fractions (particulate and associate forms) under grazed leucaena pastures. Particulate organic carbon (POC) comprises OC particles <2 mm and >53 µm in size (Cambardella and Elliot 1992). POC is biologically and chemically active and is part of the labile (easily decomposable) pool of soil organic matter. Associate organic carbon (AOC) comprises OC particles <53 µm in size, and is chemically and physically protected from microbial degradation, being more stable and persistent in the soil. The quantity and vertical distribution of OC and total nitrogen (TN) stocks, and their fractions (particulate and associate forms), in the soil profile (0–100 cm) of a 4-year-old leucaena-grass pasture, were compared with those in soil of the adjacent pure tropical grass pasture in the Chaco region of Argentina.

Materials and Methods

Site description

This study was carried out at the Animal Research Institute of the Semi-arid Chaco Region (IIACS), operated by the National Institute of Agricultural Technology (INTA), located at Leales, Tucumán (27º11' S, 65º14' W; 335 masl), in the west of the Chaco region, Northwest Argentina. The climate is subtropical sub-humid with a dry season from April to September and average annual rainfall of 880 mm (75% in October–March). Average maximum/minimum temperatures are 32/20 ºC in January and 22/7 ºC in July; on average 16 frosts occur each year, with an average ground surface temperature of -2.2 ºC and minimum temperature of -7 ºC. Mean evaporation exceeds mean rainfall in all months. Soil type is Fluvaquentic Haplustoll, US Soil Taxonomy System (Soil Survey Staff 1999).

Pasture description

The soil samples were collected from 4 parcels of 1 ha each: 2 parcels with pure grass pasture (PP) and the other 2 parcels with leucaena-grass pasture (LP). These 4 parcels had been established with a pasture of *Urochloa brizantha* (syn. *Brachiaria brizantha*) cv. Marandú (brachiaria) in 1995. In December 2009, leucaena cv. K636 was planted in 2 of these 4 brachiaria parcels, selected at random, to evaluate the effect of leucaena introduction into ageing pure grass pastures. Leucaena seed was zero till-planted in double row hedgerows (1 m apart) with 5 m between the twin hedgerows. Eight months after leucaena establishment, high grazing pressure was imposed to avoid leucaena plants growing too tall (Radrizzani and Nasca 2014), which caused a decline in grass cover and production (visual observation but not measured in this study) in the inter-row space. In December 2011, the inter-row pasture was cultivated and overseeded with *Chloris gayana* cv. Finecut (Rhodes grass) forming a brachiaria-Rhodes grass pasture. Thereafter, the high stocking rate regime continued to maintain a dense leafy canopy within browse height. Both pastures (PP and LP) have been rotationally grazed at a variable stocking rate, according to fodder availability from early spring (October) to late autumn (June). For most of the grazing periods, LP was heavily grazed with a stocking rate around 3 times that in PP in order to restrict height growth of leucaena, leading to overgrazing of the inter-row grass.

Soil sampling

Soil samples were collected in both pastures in March 2014 from 12 transects 10 m in length (3 in each parcel; 6 per pasture). In the leucaena pasture, transects were placed obliquely from leucaena hedgerows to the middle of the inter-row (2.5 m from the hedgerow) following the sampling procedure described by Radrizzani et al. (2011). Along each transect, 5 soil cores (0 to 1 m deep) divided into 3 depths: 0–20 cm, 20–50 cm and 50–100 cm, were collected at equal distances along the 2.5 m (i.e. in the leucaena pasture: 0, 0.63, 1.25, 1.88 and 2.50 m from hedgerow). The 5 soil samples collected for each depth were mixed to form 1 composite sample per depth and transect (3 and 6 composite samples per parcel and pasture, respectively). The assumption underlying the comparisons was that both the LP and PP pastures had similar soil properties before leucaena establishment. Therefore, the difference in soil fertility parameters between pastures could be attributed to the introduction of leucaena into the pure grass pasture.

Measurements and analytical techniques

Soil samples were air-dried (40 °C), and coarse fragments (>2 mm) including gravel, plant residues and roots were removed before grinding samples to pass a 2-mm sieve. Organic carbon (OC) concentration was determined by Walkey Black (Nelson and Sommers 1996). Total nitrogen (TN) concentration was determined by Kjeldahl (Bremner 1965). Fractions of OC and TN were measured in 50 g of each composite sample through particle size analysis, following the technique described by Cambardella and Elliot (1992); organic carbon associated with particles <53 µm was entrapped into clay and silt, and therefore, considered as associate organic carbon (AOC), with a similar arrangement for associate total nitrogen (ATN). Particulate organic carbon (POC) was calculated by subtracting AOC from OC, and particulate total nitrogen (PTN) was determined by subtracting ATN from TN.

Statistical analyses

Analysis of variance of soil fertility parameters (OC, POC, AOC, TN, PTN and ATN) and mean comparisons (Tukey, $P<0.05$) within pastures were performed to assess the effects of leucaena introduction. All statistical analyses were carried out using InfoStat software (Di Rienzo et al. 2016).

Results

Total organic carbon (OC)

In both pastures, stratification of OC was observed in the soil profile, with higher levels in the topsoil (0–20 cm horizon) than in the subsoil (20–50 cm and 50–100 cm horizons) (Figure 1A). This stratification was more pronounced in soil supporting PP than in soil supporting LP, since OC concentrations continued to decline with depth in PP but no differences were observed between subsoil depths in LP. In the topsoil horizon, OC concentrations were similar for LP and PP (1.25±0.05% vs. 1.31±0.06%, respectively). However, in the subsoil horizons, OC concentrations were higher for LP than for PP (0.71±0.07% vs. 0.58±0.03% in the 20–50 cm horizon; and 0.71±0.05% vs. 0.40±0.05% in the 50–100 cm horizon). For LP and PP soils, 53% and 43%, respectively, of the total OC in the first meter of soil was contained in the combined subsoil horizons (20–100 cm depth).

Particulate organic carbon (POC)

Concentrations of POC were also stratified in both pasture soil profiles but stratification was different from that for OC (Figure 1B). In contrast with OC concentrations, POC was higher in PP than in LP in the topsoil horizon (0.48±0.08% vs. 0.40±0.05%, respectively) and represented 61.5% of total POC for PP and 45.5% of total POC for LP. In the 20–50 cm horizon, POC was higher in LP than in PP (0.28±0.08% vs. 0.17±0.04%, respectively) and in the 50–100 cm horizon POC was again higher in LP than in PP (0.20±0.05% vs. 0.13±0.03%).

Associate organic carbon (AOC)

Concentrations of AOC were also stratified, but differences between pasture soils were restricted to the 50–100 cm horizon where AOC was higher in LP than in PP (0.51±0.03% vs. 0.27±0.05%, respectively) (Figure 1C). The topsoil horizon contained 47.5% of total AOC for LP and 55% of total AOC for PP.

Total nitrogen (TN)

Concentrations of TN followed a similar trend to OC (Figure 1D). However, TN was higher in LP than in PP only in the topsoil horizon (0.141±0.0039% vs. 0.131±0.0035%, respectively). In the subsoil horizon, no differences were observed between LP and PP (20–50 cm depth: 0.070±0.0030% vs. 0.069±0.0033%, respectively; and 50–100 cm depth: 0.054±0.0040% vs. 0.050±0.0038%, respectively).

Particulate organic nitrogen (PON)

Concentrations of PON were also stratified in both pasture soil profiles but followed different patterns from those for the TN concentrations in the subsoil (Figure 1E). In the 0–20 cm horizon, PON was greater in LP than in PP (0.08±0.002% vs. 0.06±0.003%, respectively), showing that most of the TN in this horizon was in the labile ON form. A similar result was observed in the 20–50 cm horizon, where PON was also higher in LP than in PP (0.04±0.001% vs. 0.02±0.001%). In contrast, PON was higher in PP than in LP in the 50–100 cm horizon (0.02±0.002% vs. 0.01±0.002%).

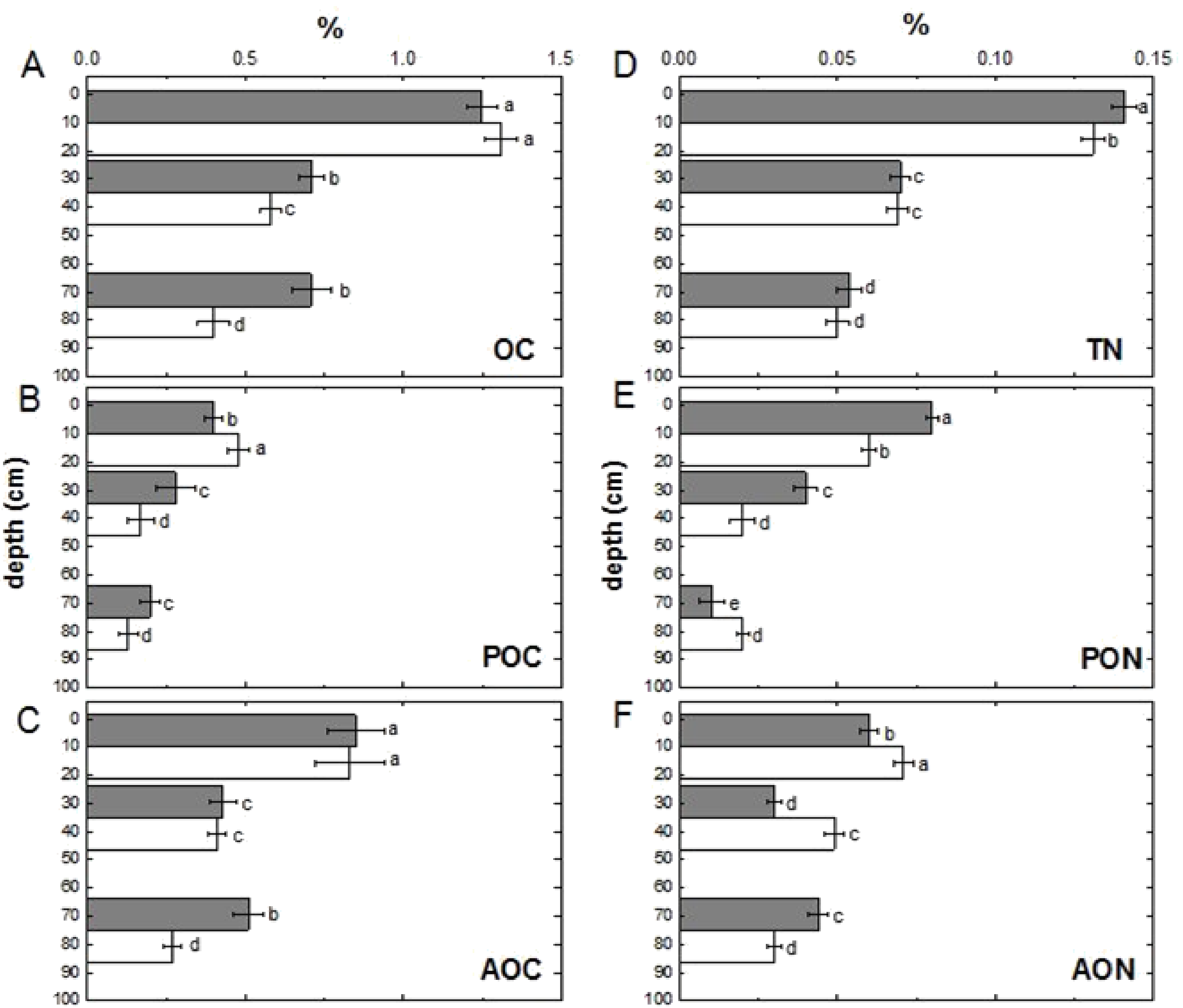

Figure 1. Concentrations of: **A**, organic carbon (OC); **B**, particulate OC (POC); **C**, associate OC (AOC); **D**, total nitrogen (TN); **E**, particulate organic nitrogen (PON); and **F**, associate organic nitrogen (AON), in relation to soil depth (0–20, 20–50 and 50–100 cm horizons) in soils under leucaena-grass pasture (filled squares) and pure grass pasture (open squares) at IIACS-INTA. Means followed by different letters are significantly different (P<0.05); bars represent standard error.

Associate organic nitrogen (AON)

Concentrations of AON were also stratified as were TN and PON but the relationships were the mirror images of those for PON (Figure 1F). AON was higher in PP than in LP in the 0–20 cm (0.071±0.003% vs. 0.06±0.002%, respectively) and 20–50 cm (0.049±0.003% vs. 0.03±0.001%) horizons. In contrast, AON was higher in LP than in PP in the 50–100 cm horizon (0.044±0.004% vs. 0.03±0.003%).

Ratios of carbon to nitrogen (OC:TN, POC:PON and AOC:AON)

The OC:TN ratio increased with depth in the LP pasture, while it decreased with depth in the PP pasture (Table 1a). While this ratio was higher in PP than in LP in the surface horizon, the reverse was the case in the 20–50 cm and 50–100 cm horizons. The POC:PON ratio also increased with depth in LP and decreased in PP (Table 1b). While the ratio was narrower in LP than in PP for the 0–20 and 20–

50 cm horizons, the reverse was the case in the 50–100 cm horizon, with a very high ratio for LP. The AOC:AON ratios declined with depth under both pastures and were higher in LP than in PP for all soil horizons (Table 1c).

Table 1. Organic carbon:total nitrogen (OC:TN), particulate organic carbon:particulate organic nitrogen (POC:PON) and associate organic carbon:associate organic nitrogen (AOC:AON) ratios in soils for leucaena-grass pasture (LP) and pure grass pasture (PP) at IIACS-INTA. Means within parameters followed by different letters are significantly different (P<0.05).

Soil depth (cm)	LP	PP
a) Mean OC:TN ± s.e.		
0–20	8.9±0.19c	10.0±0.15b
20–50	10.1±0.35b	8.4±0.32c
50–100	13.2±0.18a	8.0±0.22c
b) Mean POC:PON ± s.e.		
0–20	5.0±0.33d	8.0±0.38b
20–50	7.0±0.29c	8.5±0.68b
50–100	20.0±1.24a	6.5±0.44c
c) Mean AOC:AON ± s.e.		
0–20	14.2±0.77a	11.7±0.61b
20–50	14.3±0.81a	8.4±0.48c
50–100	11.6±0.55b	9.0±0.38c

Discussion

This study generated data from a real grazing system that described the effects on soil properties of the introduction of a forage tree legume into a tropical grass pasture. However, a lack of an accurate baseline measurement of the initial pasture soil properties did prevent rigorous statistical comparison before and after leucaena introduction. Results demonstrated the increase in OC concentration in the subsoil (20–100 cm depth), particularly the stable OC form (AOC) in the deepest horizon (50–100 cm), 4 years after leucaena introduction into a grass pasture. Results also showed that the introduction of leucaena enhances the TN in the topsoil associated with an increment of the labile ON form (PON).

Changes in organic carbon and its fractions

Overall, OC concentrations for leucaena-grass pastures were similar to values reported by Banegas (2014) in similar soil types of the same area. The 43% of the total OC in the subsoil (20–100 cm depth) of the pure grass pasture was similar to the percentages reported by Banegas (2014) in grazed pure grass pastures in the same area and by Babujia et al. (2010) in Brazilian oxisols with tropical grass pastures. Within 4 years from planting, leucaena increased the percentage of total OC contained in the subsoil from 43% to 53%, and most of this OC was in the most stable form (AOC). Similar increments in subsoil OC have been reported by Carter et al. (1998) in leucaena and *Stylosanthes* spp. pastures in northern Australia; 10 years after the woody forage legumes were introduced, they had accumulated more OC in the 20–65 cm soil horizon than the adjacent native grass pastures. Comparable results were observed 9–16 years after *Desmodium ovalifolium* (Tarré et al. 2001) and *Stylosanthes capitata* and *Arachis pintoi* (Fisher et al. 1994) were oversown into tropical grass pastures.

Although it is known that leucaena establishment can enhance OC concentrations in topsoil via its N contribution, which increases tropical grass growth, litter recycling and humus formation (Radrizzani et al. 2011; Conrad et al. 2017), in this study the most labile C form (POC) was lower in topsoil for LP than for PP. This unexpected result could be mainly attributed to two causes: a) the higher stocking rate imposed on the leucaena-grass pasture in comparison with the pure grass pasture (Radrizzani and Nasca 2014) caused overgrazing and a decline in the inter-row grass cover (visual observation but not measured in this study), hence reducing grass litter deposition and grass root turnover; and b) the cultivation done in December 2011 (only in LP pasture) might have accelerated mineralization of the labile OC form (POC) and 2.5 years might have been insufficient time to recover the original value (POC in PP pasture) in the inter-row area.

The high concentration of the stable OC form (AOC) in the deepest horizon (50–100 cm) is consistent with studies that show that root turnover in deep soil enhances the pool of less-labile soil OC (Fisher et al. 1994; Follett et al. 2003). This increment of stable C in the subsoil could be attributed to leucaena's deep-root system (not measured in this study), since a larger proportion of fine roots (>60%) of leucaena have been observed below 40 cm in soil compared with the adjacent grass pastures (Radrizzani 2009). Pachas et al. (2018) determined abundance of roots of leucaena and Rhodes grass (*Chloris gayana*) and found that leucaena had a greater abundance of roots deeper in the profile than the grass. Moreover, it is known that defoliation promotes significant turnover of fine roots (Jayasundara et al. 1997; Franzluebbers and Stuedemann 2005). Root carbon turnover would have contributed significantly to the increment in subsoil OC, particularly under the high stocking rate applied in this leucaena pasture (Radrizzani and Nasca 2014).

Although sampling up to 3 or 4 m depth is recommended to assess OC in systems where shrubs or trees grow (Jobbágy and Jackson 2000), in this study available funds did not permit collecting deeper soil samples. Consequently, the sampling depth to a meter underestimated the OC concentrations, particularly in the leucaena pasture where the OC concentration did not decline from 20 to 100 cm (Figures 1A, 1B and 1C); additional soil OC might be accumulated below the top meter. Therefore, in further surveys of silvopastoral systems soil samples should be collected to a depth of at least 3–4 meters to take account of the whole OC contribution from leucaena.

Changes in total nitrogen and organic nitrogen fractions

Overall, soil TN concentrations and organic nitrogen fractions for leucaena-grass pastures and grass pastures were within the range reported in the same area by Banegas (2014), Corbella et al. (2015) and Conrad et al. (2018). Like soil OC concentrations, soil TN declined with depth in both pastures, since most of the N (~90%) was bound up with OC in organic matter. However, in leucaena pasture, TN did not follow the same trend as soil OC concentrations, associated with the great increase in the labile PON form in both the 0–20 cm and 20–50 cm horizons. This result is consistent with higher soil TN concentrations in topsoil (0–15 cm) reported by Radrizzani et al. (2011) and Conrad et al. (2018) in leucaena pastures in comparison with adjacent pure grass pastures in northern Australia. A similar result was reported by Mahecha et al. (1999), who observed significant increases in TN concentration in topsoil (0–20 cm) of leucaena silvopastoral systems relative to pure grass pastures in the Valle del Cauca region, Colombia.

The increase in N concentration in the topsoil of leucaena-grass pasture, mainly in the labile PON form, could be attributed to deposition of leucaena leaf which is high in N (e.g. frost causes leucaena leaf shedding), leaf recycling via animal feces (e.g. high grazing pressure) and nodule-N turnover from biological N fixation. Grazing management (e.g. rotational, seasonal or continuous grazing) and weather conditions (e.g. frost and drought) can influence the quantities and the proportions of leucaena leaf fall and leaf recycled via dung (Burle et al. 2003). In the 20–50 cm horizon, the greater proportion of PON compared with AON could be attributed mainly to nodule-N turnover from biological N fixation.

Changes in carbon:nitrogen ratios

Overall, soil C:N ratios for leucaena-grass pastures and grass pastures were within the range reported in the same area by Banegas (2014) and Corbella et al.(2015). Results showed that C:N ratio increased with depth in the leucaena pasture but decreased with depth in the grass pasture, showing inverse relationships between C:N ratio and soil depth for the 2 pastures. In the leucaena-grass pasture, higher inter-row grass production and quality than in the pure grass pasture could be expected. It is known that biomass production of pure grass pastures is limited due to soil N being immobilized in litter and soil organic matter (Graham et al. 1981; Robbins et al. 1989). The increase in TN of topsoil via deposition of N-rich leaf and biological N fixation by leucaena might enhance available N for grass growth, leading to an increase in inter-row grass yield and quality.

In relation to the ratios of various parameters, the main contribution of leucaena was in the PON (labile N form) in the top 50 cm of the LP soil profile (0–20 and 20–50 cm horizons), with lower POC:PON ratios than in the deeper soil (50–100 cm). Similar findings were reported by Luce et al. (2013) with significant increases in the labile fractions of N attributed to both recycling of N-rich residues and biological N fixation after legume introductions; they highlighted that PON is the N form most sensitive to management-induced changes and has the potential to predict N availability for plant growth. Furthermore, Griffin and Porter (2004) showed that the inclusion of red clover as a cover crop in 2-year potato (*Solanum tuberosum*) rotations increased the proportion of total soil N as PON by 1,320% compared with rotations that did not contain a legume cover crop. In the deepest soil horizon (50–100 cm) of the leucaena-grass pasture, POC:PON ratio was considerably higher than in other treatments associated with the high POC concentration that might be formed by deep roots of leucaena.

The high ratios of the associate fractions (AOC:AON) in the topsoil of both pastures and the low variability with depth of these ratios, is consistent with the high stability of the associate fractions.

Conclusions

Introduction of leucaena into a grass pasture promoted substantial capture of OC in the subsoil (20–100 cm), especially the most stable form (AOC), which has minimal susceptibility to mobilization in the deepest horizon (50–100 cm), attributed to a greater abundance of leucaena roots deeper in the soil profile than of the grass.

Leucaena introduction also enhanced N concentration in the topsoil (0–20 cm), particularly the most labile form (PON) that promotes improvement in grass growth and quality, attributed to N-rich leucaena leaf deposition, leaf recycled via animal feces and nodule-N turnover from biological N fixation.

Accordingly, the establishment of hedgerow leucaena silvopastoral systems can increase cattle production directly through the diet, as well as improving soil fertility and hence availability of N to companion grasses. Through the increased growth rates of animals and greater production per head and per unit area, this strategy can serve as a long-term greenhouse gas mitigation strategy.

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(Note of the editors: All hyperlinks were verified 8 August 2019.)

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