Isotopic δ^{15}N Signature of Grass-Alone and Grass-Legume Tropical Pastures to Estimate Sources of Nitrogen to Grasses in Farmer Managed Pastures

Daniel M. Villegas
International Center for Tropical Agriculture, Colombia

J. Velasquez
Universidad de la Amazonia, Colombia

J. Arango
International Center for Tropical Agriculture, Colombia

K. Obregon
Universidad de la Amazonia, Colombia

Idupulapati M. Rao
International Center for Tropical Agriculture, Colombia

See next page for additional authors

The XXIV International Grassland Congress / XI International Rangeland Congress (Sustainable Use of Grassland and Rangeland Resources for Improved Livelihoods) takes place virtually from October 25 through October 29, 2021.

Proceedings edited by the National Organizing Committee of 2021 IGC/IRC Congress

Published by the Kenya Agricultural and Livestock Research Organization

Presenter Information

Daniel M. Villegas, J. Velasquez, J. Arango, K. Obregon, Idupulapati M. Rao, G. Rosas, and A. Oberson

Isotopic $\delta^{15}N$ signature of grass-alone and grass-legume tropical pastures to estimate sources of nitrogen to grasses in farmer managed pastures

Villegas, D. M.*; Velasquez, J.†; Arango, J.*; Obregon, K.†; Rao, I. M.*; Rosas, G.†; and Oberson, A‡.
* International Center for Tropical Agriculture (CIAT), Cali, Colombia; † Universidad de la Amazonia, Florencia, Colombia; ‡ ETH Zurich, Institute of Agricultural Sciences, Lindau, Switzerland.

Key words: Biological nitrogen fixation; *Urochloa* pastures; ^{15}N natural abundance.

Abstract

Vast areas of tropical forest have been converted into pastures sown with introduced grasses (*Urochloa* spp.). The majority of pastures exist in some stage of degradation, which has dramatic ecological and economic consequences. Our objective was to determine isotopic ^{15}N signature of grass alone and grass-legume pastures to estimate sources of nitrogen (N) to grasses in farmer managed pastures. This work contributes to enhancing the sustainability of tropical pastures in forest margins through the integration of legumes. The overarching hypothesis tested was that legumes associated with grasses will improve nutrient supply via symbiotic fixation and mobilization of soil phosphorus (P) reserves increasing belowground inputs and recycling of carbon (C), N and P. We have done preliminary research in ten paired plots of productive grass-alone vs. grass-legume pastures on farms located in the Caquetá region of Colombia. Pasture productivity and sources of plant N uptake using ^{15}N isotope natural abundance methods have been analysed. The integration of legumes increased biomass production by about 74%, and N and P uptake by two-fold. The legumes derived about 80% of their N via symbiotic fixation, showing the significance of this process to N nutrition despite the acidic soil conditions. The isotopic ^{15}N signature of grasses in grass-alone vs. grass-legume pastures suggested that sources of grass N are affected by pasture composition. Furthermore, low $\delta^{15}N$ found in some grass-alone pastures, indicate that other N sources apart from soil mineral N are being exploited. The role of different processes like symbiosis with arbuscular mycorrhiza fungi, biological nitrification inhibition or associative N fixation need to be further studied to provide a more comprehensive interpretation of N exploitation in grass-alone pastures.

Introduction

Low-productive, extensive livestock systems in the Amazon region have increased the need for land to establish new pastures and therefore have intensified the pressure over tropical rainforest (Hansen et al. 2013). Most pastures in the tropics established from former forest currently show some stage of degradation (Jimenez and Lal 2006), which has major economic and environmental consequences.

Livestock production in the Amazon region of Colombia is largely based on open grazing of monoculture of pasture grasses of the genus *Urochloa* (syn. *Brachiaria*). Degradation of such pastures has been mainly related to reduced N and P availability, and overgrazing (Boddey et al. 2004).

Association of grasses with legumes is an alternative to grass-alone pastures to provide additional nutrient inputs via N_2 fixation and recycling of organic matter. However, despite the benefits of the incorporation of legumes into pastoral systems have been widely demonstrated, adoption remains low (Schultze-Kraft et al. 2018).

Grasses growing alone, or in association with legumes may exploit different sources of N. Different mechanisms of nutrient acquisition and utilization have been reported for *Urochloa* grasses that may affect the $\delta^{15}N$ signature of its biomass, namely, associative N_2 fixation and suppression of soil nitrification (Reis et al. 2001; Subbarao et al. 2009).

Despite the benefits of grass-legume associations for productive and environmental purposes have been studied under controlled conditions, such interaction has been seldom evaluated under farmers´ pasture management practices. Here we analyze data from Villegas et al. (2020) with the aim to determine the isotopic ^{15}N signature of grass alone and grass-legume pastures to estimate sources of N to grasses in farmers´ fields in the Amazon region of Colombia.

Methods and Study Site

The experiment was carried out in farms of the Caquetá department of Colombia, located in the Amazon piedmont of the eastern Andean region. Ten paired grass-alone and grass-legume plots in neighbour pastures were selected in six farms, which were between 16 to 32 years of age after establishment.

Within each plot, in May 2019, a 5 m × 5 m area was delimited and fenced to avoid animal intervention, 1 m^2 of plant aboveground biomass was cut to soil level and let to recover for 45 days. After the recovery period of the pasture, plant living material was harvested and split into four types: principal grass, secondary grass, legumes, and forbs. A separate plant litter sample was also collected. Soil (0–10 cm depth) was sampled from different points inside the plot and a composite sample was analysed. Grasses evaluated in the farms were *Urochloa humidicola*, *U. brizantha*, and *U. decumbens* alone or in association with forage legumes of either *Arachis pintoi* or *Pueraria phaseoloides*. Plant samples were oven-dried at 60°C for 72 h to determine dry matter content and were ground to a fine powder for analysis. Soils were air-dried for 48 h and sieved to 2 mm particle size.

Plants and soils were analysed for total N, and $^{15}N/^{14}N$ isotopic ratio by dry combustion using an NCS elemental analyser coupled to an Isotope Ratio Mass spectrometer (Vario PYRO cube, Elementar, Germany and IsoPrime100 IRMS, Isoprime, United Kingdom) at ETH Zurich, Eschikon, Switzerland. Total P concentration in plant tissue was determined by microwave digestion with nitric acid and colorimetric determination by the malachite green method (Ohno and Zibilske 1991). N and P uptake per plant type were calculated multiplying the individual nutrient concentration by the biomass production per m^2. Total nutrient uptake was determined adding the nutrient uptake of each botanical fraction except plant litter in 1 m^2. The weighted $\delta^{15}N$ of the pastures (on a m^2 basis) was determined multiplying the $\delta^{15}N$ of each plant type by their N uptake, and the final result was divided by the total N uptake of the pasture.

The N derived from the atmosphere (%Ndfa) was determined by the ^{15}N natural abundance method described by Unkovich et al. (2008). Forbs growing in the grass-alone plots were used as non-N-fixing reference plants.

The data were analyzed by linear mixed-effect models considering pasture type and plant type as fixed factors and farm as a random effect. All statistical analysis was performed in R v3.4.4.

For further details on the methods and formulas used for calculations see Villegas et al. (2020).

Results

Plant biomass production and nutrient uptake

The association of grass-legume pastures produced 74% more plant biomass and showed two times greater N and P uptake compared to the grass-alone pastures (Table 1). In grass-legume pastures, legumes showed twice the N concentration observed in grasses, but no difference was observed for P concentration in shoot tissue. Weighted N concentration of grass-legume association was significantly higher than that of grass-alone pasture. Such results were observed with an average grass-legume ratio of 65:35 of the total biomass found in the grass-legume pastures. Lower abundance of forbs (undesirable species) was found in the grass-legume than the grass-alone pastures. The nutrient concentration of plant litter was similar among both pasture types (results not shown).

Table 1. Plant biomass production, nutrient uptake and N isotopic signature in grass-alone and grass-legume pastures. Values in parentheses represent the standard deviation of the mean, n = 10 for grass-alone and n = 8 for grass-legume pastures. Different letters denote statistical differences according to the TukeyHSD test (α = 0.05).

Pasture type	Dry matter productivity (kg m^{-2})	N uptake (g m^{-2})	P uptake (g m^{-2})	Total N in principal	Total N in legume (g kg^{-1})	Weighted $\delta^{15}N$ (‰)

				grass (g kg^{-1})		
Grass-alone	62.4 (43.2)a	0.8 (0.5)a	0.07 (0.05)a	14.9 (3.5)a	-	4.6 (2.9)a
Grass-legume	107.8 (41.8)b	2.2 (1.3)b	0.14 (0.08)b	14.8 (3.5)a	27.8 (3.3)	2.1 (1.7)b

Legume-N derived from the atmosphere
Two legume species were identified in the evaluated farms: *Pueraria phaseoloides* and *Arachis pintoi*. The calculated %Ndfa for *P. phaseoloides* was 83.7%, while it was 67.5% for *A. pintoi*. The amount of N fixed in legumes´ biomass was estimated up to 3.7 g N m^{-2}.

Isotopic δ^{15}N signature of pasture components
The weighted δ^{15}N of the combined biomass of the grass-alone swards was 4.6‰, and it was 2.1‰ for grass-legume swards. The δ^{15}N signature of the soil or separate plant types was not statistically different between both pasture types. Significant differences were observed in the δ^{15}N signature among plant species (Figure 1). For the grasses, the highest δ^{15}N signature observed was that of *U. decumbens*, followed by *U. brizantha* and *U. humidicola*. Regarding legumes, the δ^{15}N signature of *P. phaseoloides* was significantly higher than that of *A. pintoi*. The δ^{15}N signature of grasses was highly variable, ranging from −1.2 to 8.9‰. Variation was higher in grass-alone than in grass-legume pastures, and higher in *U. humidicola* compared to the other grass species.

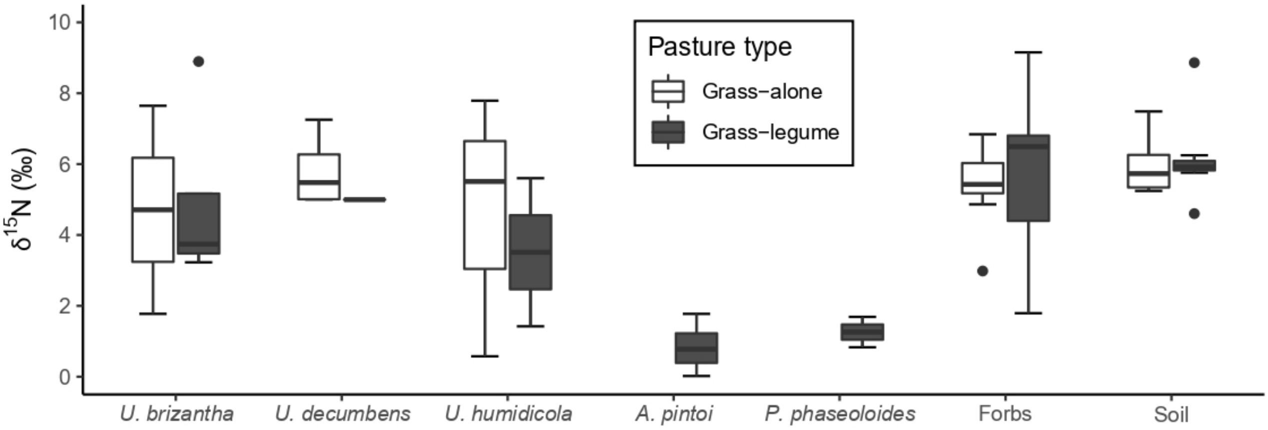

Figure 1. δ^{15}N signature of principal grasses, legumes, forbs and soil in grass-alone and grass-legume pastures.

Discussion
Association of grass-legume pastures outperformed grass-alone pastures both in terms of plant biomass production, and nutrients uptake. The weighted δ^{15}N of grass-legume pastures was significantly lower than that of grass-alone pastures, and N fixation was estimated at around 80% by associated legumes. A legume proportion of 20 to 45% of total dry matter has been estimated to cover N requirement of pasture growth in the tropics (Thomas 1992), being animal excreta and litter decomposition the main N transfer pathways (Dubeux et al. 2015). Our findings suggest that grasses in the grass-legume plots might have benefited from atmospheric-N fixed by legumes to cover N demand.

Variation of the δ^{15}N signature of grasses was higher in the grass-alone than in grass-legume pastures. This may indicate that grasses exploit different N sources when they are cultivated alone, than when associated with legumes. The capacity of certain tropical grasses to obtain atmospheric N has been well documented (Reis et al. 2001), and it is known that *Urochloa* grasses may obtain around 20% of N from the atmosphere, a process that may alter the δ^{15}N signature of its biomass. On the other hand, low δ^{15}N signatures have been also related to biological nitrification inhibition (BNI) in *U.*

humidicola (Karwat et al. 2018), a strategy that presumably conserves N in ammonium form in the soil suppressing the activity of microbial nitrifiers (Subbarao et al. 2009).

Grass-legume associations are an alternative to grass-alone pastures towards more sustainable livestock production models while preventing pasture degradation. To elucidate the specific N sources used by grasses depending on pasture composition, the role of BNI, associative N fixation, and mycorrhizal association should be further studied.

Acknowledgements
The authors acknowledge the financial support of the Leading House for the Latin American Region, University of St. Gallen, Switzerland. We acknowledge the UK Research and Innovation (UKRI) Global Challenges Research Fund (GCRF) *GROW Colombia* grant via the UK's Biotechnology and Biological Sciences Research Council (BB/P028098/1) to D.V. and J.A. Additional funding for this study was received from the CGIAR Research Programs on Livestock; and Climate Change, Agriculture and Food Security (CCAFS). We warmly acknowledge the great cooperation of farmers in the region. We thank Dr. Federica Tamburini (ETH Zurich) for mass spectrometry measurements.

References

Boddey, R.M., Macedo, R., Tarre, R.M., Ferreira, E., de Oliveira, O.C., Rezende, C.D., Cantarutti, R.B., Pereira, J.M., Alves, B.J.R. and Urquiaga, S. 2004. Nitrogen cycling in *Brachiaria* pastures: the key to understanding the process of pasture decline. *Agriculture Ecosystems & Environment*., 103: 389-403.

Dubeux, J.C.B., Muir, J.P., Nair, P.K.R., Sollenberger, L.E., Silva, H.M.S. and Mello, A.C.L. 2015. The advantages and challenges of integrating tree legumes into pastoral systems. In: Evangelista, A.R., Avila, C.L.S., Casagrande, D.R., Lara, M.A.S. and Bernardes, T.F. (eds). *Proceedings of the 1st International Conference on Forages in Warm Climates*. Universidade Federal de Lavras, Lavras, MG, Brazil, pp. 141−164.

Hansen, M.C., Potapov, P.V., Moore, R., Hancher, M., Turubanova, S.A., Tyukavina, A., Thau, D., Stehman, S.V., Goetz, S.J., Loveland, T.R. and Kommareddy, A. 2013. High-resolution global maps of 21st-century forest cover change. *Science*., 342: 850-853, doi:10.1126/science.1244693.

Jimenez, J.J., and Lal, R. 2006. Mechanisms of C sequestration in soils of Latin America. *Critical Reviews in Plant Sciences*., 25: 337-365, doi:10.1080/0735268060094240.

Karwat, H., Egenolf, K., Nuñez, J., Rao, I., Rasche, F., Arango, J., Moreta, D., Arevalo, A. and Cadisch, G. 2018. Low ^{15}N natural abundance in shoot tissue of *Brachiaria humidicola* is an indicator of reduced N losses due to biological nitrification inhibition (BNI). *Frontiers in microbiology*., 9: 2383.

Ohno, T., and Zibilske, L.M. 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Sci Soc Am J*., 55: 892-895.

Reis, V.M., dos Reis, F.B., Quesada, D.M., de Oliveira, O.C.A., Alves, B.J.R., Urquiaga, S. and Boddey, R.M. 2001. Biological nitrogen fixation associated with tropical pasture grasses. *Aust. J. Plant Physiol.*, 28: 837-844, doi:10.1071/pp01079.

Schultze-Kraft, R., Rao, I.M., Peters, M., Clements, R.J., Bai, C., and Liu, G. 2018. Tropical forage legumes for environmental benefits: An overview. *Tropical Grasslands-Forrajes Tropicales*., 6: 1-14.

Subbarao, G.V., Nakahara, K., Hurtado, M.P., Ono, H., Moreta, D.E., Salcedo, A.F., Yoshihashi, A.T., Ishikawa, T., Ishitani, M., Ohnishi-Kameyama, M. and Yoshida, M. 2009. Evidence for biological nitrification inhibition in *Brachiaria* pastures. *Proceedings of the National Academy of Sciences of the United States of America*., 106: 17302-17307, doi:10.1073/pnas.0903694106.

Thomas, R.J. 1992. The role of the legume in the nitrogen cycle of productive and sustainable pastures. *Grass and Forage* Science., 47: 133-142.

Unkovich, M., Herridge, D., Peoples, M., Cadisch, G., Boddey, R., Giller, K., Alves, B., and Chalk, P.M. 2008. *Measuring plant-associated nitrogen fixation in agricultural systems*; ACIAR: Canberra, Australia, 136.

Villegas, D.M., Velasquez, J., Arango, J., Obregon, K., Rao, I.M., Rosas, G.,and Oberson, A. 2020. *Urochloa* grasses swap nitrogen source when grown in association with legumes in tropical pastures. *Diversity*., 12(11): 419.