Scientific Paper

Synergistic effect of biofertilizer and nitrogen fertilizer application in pastures

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Abstract

Objective: To evaluate the synergistic application of biofertilizer and water-soluble fertilizer on the yield and quality of pastures under the soil and climate conditions of Tumbaco-Ecuador.

Materials and Methods: Biofertilizer produced by facultative anaerobic fermentation of dairy cow effluents was used. Three doses of biofertilizer (0, 600; 1 200 L ha^{-1} cut^{-1}) and three doses of water-soluble nitrogen fertilizer (0; 10; 20 kg N ha^{-1} cut^{-1}) were evaluated. A complete randomized block design was used, with three blocks and nine treatments. The variables biomass, crude protein and normalized difference vegetation index were analyzed. Seven cuts were made from January to July.

Results: The best results were obtained when nitrogen fertilizer and biofertilizer were used in combination. Statistical differences ($p < 0,05$) were observed in the studied variables. The treatment with 600 L and 20 kg N ha^{-1} cut^{-1} recorded the highest biomass yield with 3 177 kg dry matter ha^{-1}. Crude protein indicated values of 21,6 % and the normalized differentiated vegetation index reached values of 0,89 when 1 200 L ha^{-1} cut^{-1} and 20 kg N ha^{-1} cut^{-1} were used.

Conclusions: Biofertilizers have low nutrient concentration, but great potential, since they improve the assimilation of nitrogen fertilizers and good yields and pasture quality are achieved with relatively low doses of nitrogen between cuts.

Keywords: quality, animal husbandry, production

Introduction

The productivity of a crop is determined by the interaction of the crop's genetic potential, environmental factors and management (Nair, 2019). However, nitrogen (N) is an essential nutrient and as genetic improvement has increased the yield potential of crops, its demand grows. Therefore, nitrogen fertilization becomes a determinant of yield variation (Hoffman *et al*., 2016).

Nitrogen fertilization is a necessary management practice for sustainable agriculture. However, given the cost of nitrogen fertilizers and the environmental impact resulting from their application it is necessary to develop management strategies to improve N use efficiency (Barbieri *et al*., 2010). Many times the intensive use of chemical fertilizers exceeds the thresholds required by crops and soils, with the aim of increasing yields (Liu *et al*., 2017).

With the development of the synthetic N fixation method and the obtaining of nitrogen fertilizers from natural gas, the use of legumes as the main source of N for productive systems was replaced (Escobar *et al*., 2020).

In that sense, sustainable agriculture is an agricultural practice that promotes soil health, increases agricultural yields, and reduces pollution of arable soils. From an ecological point of view, soil is a dynamic habitat for a huge variety of life forms (Wiesmeier *et al*., 2019).

In this regard, Etesami, (2020) indicates that fertilizer use efficiency is improved when beneficial soil microorganisms are used. The abundance of soil macrofauna favors higher production and lower environmental pollution in agrosystems, mainly due to increased soil quality (Sofo *et al*., 2020).

According to Odoh *et al*. (2020), the use of biofertilizers, microbial formulations in organic carrier materials that improve soil health, crop growth and development, has gained much acceptance. Soil management is the foundation of agriculture and is essential for sustainable forage production. Therefore, pasture fertilization decisions should include production as well as conservation goals (Silveira and Kohmann, 2020). Panpatte and Jhala (2019) argue that soil fertility requires agronomic and microbiological strategies.

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Meanwhile, Maurya *et al*. (2020) mention that soil has physical, chemical and biological components. All of them are involved in its functioning.

The objective of this research was to evaluate the synergistic application of biofertilizers and water-soluble fertilizers on the yield and quality of pastures under the soil and climate conditions of Tumbaco-Ecuador.

Materials and Methods

Location. The study took place at the Academic Teaching and Experimental Campus La Tola (CADET, for its initials in Spanish), of the School of Agricultural Sciences (FCAg, for its initials in Spanish) of the Central University of Ecuador (UCE, for its initials in Spanish), located in the Tumbaco parish, Quito canton, Pichincha province, at 2 465 m.a.s.l. 00° 14' 46 "S, 78° 22' 00"W.

Treatments and experimental design. A complete randomized block design was applied, with three blocks and nine treatments (table 1). The experimental unit consisted of 6 x 4 m plots.

Table 1. Doses of biofertilizer and nitrogen fertilizer (ammonium nitrate).

Treatment	Biofertilizer L ha^{-1} cut^{-1}	Nitrogen fertilization kg N ha^{-1} cut^{-1}
T1	600	0
T2	1200	0
T3	0	10
T4	0	20
T5	600	10
T6	600	20
T7	1 200	10
T8	1 200	20
T0	0	0

Edaphoclimatic characteristics. The average rainfall of the study site was 952 mm and temperature was 16,4 °C (INAMHI, 2022). A soil survey was conducted at the beginning of the experiment and after seven months of application of the factors under study (table 2).

Experimental procedure. Three doses of biofertilizer (BF) (0; 600; 1 200 L ha^{-1} cut^{-1}) and three doses of nitrogen fertilizer (NF) (0; 10; 20 kg N ha^{-1} cut^{-1}) plus an absolute control were evaluated in the experiment. The fertilizer and biofertilizer were applied seven days after cutting. The N source used was ammonium nitrate. Supplemental amounts of phosphorus, potassium, magnesium, sulfur and the microelements boron and zinc were also supplied, according to crop requirement and soil analysis. The nutrients were applied at planting time.

The BF was obtained by facultative anaerobic fermentation of waste (manure and urine) from the barn at the time of milking. A pool was constructed for facultative anaerobic fermentation and another one for biofertilizer collection (fig. 1). The characterization of the used biofertilizer is shown in table 3.

Plots were established with a forage mixture composed of 110 g of *Lolium perenne* L. (perennial ryegrass), 35 g of *Cichorium intybus* L. (chicory) and 35 g of *Trifolium pratense* L. (white clover). They were sown in furrows, separated by a distance of 10 cm and at a depth of 2 cm. Seven cuts were evaluated, with an interval of 28 days, time in which physiologically the pasture in the agroclimatic conditions of the experiment reaches its maximum growth point, without the beginning of leaf senescence (Berone, 2016). The plots were maintained at field capacity with a sprinkler irrigation system. The experiment was conducted between January and July.

Measurements. Biomass production, protein (CP) and normalized vegetation index (NDVI)

Table 2. Soil analysis at a depth of 20 cm.

Indicator	pH	SOM	Total, Nitrogen	P	K
		%	%	ppm	ppm
Initial analysis	7,31	0,6	0,03	34,2	0,31
Nitrogen fertilizer	6,62	1,59	0,08	43,9	1,55
Nitrogen fertilizer x biofertilizer	6,69	2,31	0,12	24,2	1,32
Biofertilizer	6,95	2,66	0,13	32,7	1,56
Control	7,23	0,92	0,04	27,5	0,95

SOM: Soil organic matter

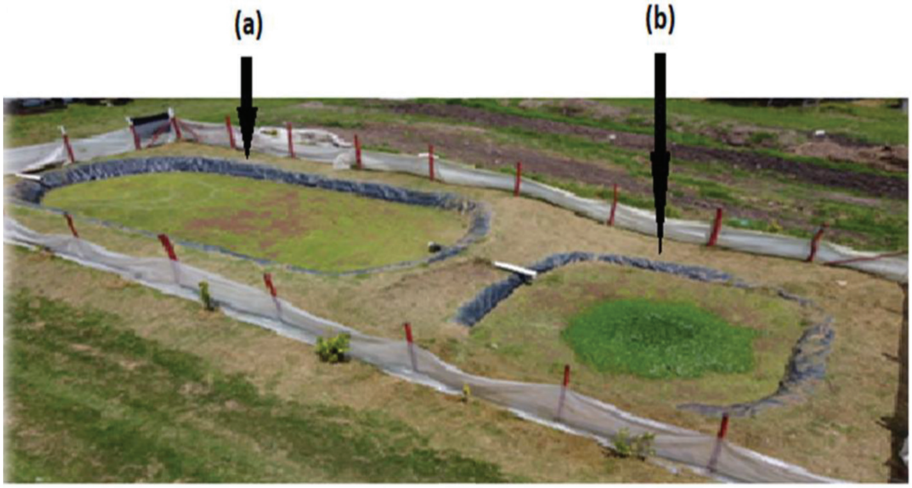

Figure 1. Facultative anaerobic fermentation pools; (a) treatment of stable wastewater (urine and feces); (b) biofertilizer.

Table 3. Nutrient content, electrical conductivity and pH of the biofertilizer.

pH	EC	N	P	K	Ca	Mg	Fe	Mn
	µS cm^{-1}				(mg L^{-1})			
7,38	1,01	45	17	232,7	32,5	18,9	0,91	0,41

variables were evaluated. Biomass production was determined by the quadrat method (0,3 x 0,3 m) and plots were randomly sampled. All green matter was collected by a shallow cut. Dry matter (DM) content was determined and total production per hectare (kg DM ha^{-1}) was calculated according to the methodology proposed by Hall (2009).

Protein analysis was performed on the same DM sample, ground and sieved on a 750-µm mesh using the semi-micro Kjeldahl method (official reference method AOAC 2001.11). The result was expressed as percentage (Thiex *et al*., 2002).

The GreenSeeker equipment was used to determine the NDVI. The sensor employs a technology to measure crop reflectance and calculate NDVI:

$$NDVI = (\rho\ RIC - \rho\ Red)/(\rho\ RIC + \rho\ Red).$$

Where ρ RIC is the fraction of emitted near-infrared radiation returned from the sensed area (reflectance), and ρ Red is the fraction of emitted red radiation returned from the sensed area (reflectance). These data are used in an algorithm and a value between 0 and 1 is determined (Walsh *et al*., 2013).

Statistical analysis. Variance analysis was performed after testing the assumptions of homoscedasticity with Levene's test and normality with the modified Shapiro-Wilks test. Tukey's test was applied for the separation of means of the variables that indicated statistical significance ($p < 0,05$) among treatments. The INFOSTAT program was used.

Results and Discussion

Biomass. For the BF factor, no statistical differences were found among treatments. However, the NF factor showed statistical differences ($p < 0,05$). The treatments with 10 and 20 kg N ha^{-1} cut^{-1} were those with the highest biomass yield (2 515 and 2 688 kg DM ha^{-1} cut^{-1}, respectively) with respect to 0 kg N ha-1 cut-1 (1 745 kg DM ha^{-1} cut^{-1}).

The interaction between BF x NF showed statistical differences ($p < 0,05$) and indicated three ranges of significance: the highest range was associated with 20 kg N ha^{-1} cut^{-1} and 600 L of BF ha^{-1} cut^{-1}reaching 3 177 kg DM ha^{-1} cut^{-1}. The lowest was reached when only 600 L of BF ha^{-1} cut^{-1} and the control were applied, with yields of 1 647 and 1 542 kg DM ha^{-1} cut^{-1}, respectively (table 4).

Orozco-Corral *et al.* (2016) point out that organic fertilizers are an alternative to replace inorganic fertilization. This is due to the fact that these fertilizers supply the soil not only with a single nutrient, as in this case N, but they also provide other essential elements. In addition,

Table 4. Effect of biofertilizer and nitrogen fertilizer on biomass.

Factor	Dose	kg DM ha^{-1} cut^{-1}	P - value
Biofertilizer	0	1 682	0,159
	600	1 946	
	1 200	1 862	
Nitrogen fertilizer	0	1 745^{b}	0,0001
	10	2 515^{a}	
	20	2 688^{a}	
	Interaction		
Biofertilizer	Nitrogen fertilizer	kg DM ha^{-1} cut^{-1}	P - value
600	20	3 177^{a}	0,0459
1 200	20	2 590^{ab}	
600	10	2 566^{ab}	
0	10	2 548^{ab}	
1 200	10	2 432^{ab}	
0	20	2 297^{bc}	
1 200	0	2 048^{bcd}	
600	0	1 647^{cd}	
0	0	1 542^{d}	
Mean		2 081	
VC, %		30,0	

Different letters in the same column indicate significant differences between treatments ($p < 0{,}05$).

biofertilizers can influence the synthesis of growth regulators, such as auxins and gibberellins, which produce an increase in root hair development and density, providing the plant with greater viability, productivity and resistance to adverse conditions such as drought (Saharan and Nehra, 2011).

Wallace *et al.* (2009) observed that biofertilizer application improves soil carbon (C) storage, water retention, nutrient availability, and ultimately the overall health of perennial grasses. The combined use of biofertilizers and fertilizers can reduce C footprint by 17 % compared with fertilizer use alone (Neves *et al*., 2017).

According to Tilman *et al.* (1996), one factor that is related to soil fertility, grassland production and quality is species association. For these authors, the sustainability of soil nutrient cycles and, therefore, fertility depends on biodiversity. Furey and Tilman (2021) suggest that soil C reserves and soil fertility improve with plant diversity. It was found that when grasses, legumes and other species are present, significantly more N, K, Ca and Mg accumulate in the total nutrient pool (plant biomass and soil) with regards to plots containing only one of these three functional groups. Castro *et al.* (2009) reported that animals grazing grass and legume associations showed better efficiency in the use of nitrogen fertilizers than those grazing mixed grass pastures fertilized with nitrogen.

Crude protein. The CP analysis indicated statistical differences ($p < 0{,}05$) for BF. With applications of 600 and 1 200 L ha^{-1} cut^{-1} 18,5 and 19,2 % of CP were achieved respectively, and 17,8 % when BF was not applied. Statistical analysis also showed statistical differences ($p < 0{,}05$) for NF. The highest value (19,9 % of CP) was obtained with the dose of 20 kg N ha^{-1} cut^{-1}, followed by 10 kg ha^{-1} cut^{-1} with 18,4 %. The CP concentration decreased to 17,2 % when BF was not applied. The interaction of BF x NF was significant ($p < 0{,}05$). Four ranges of significance were obtained. The highest concentration of CP (21,6 %) was recorded when 1 200 L ha^{-1} cut^{-1} and 20 kg N ha^{-1} cut^{-1} were applied compared with the lowest CP values (16,9 and 16,5 %), associated with 600 L ha^{-1} cut^{-1} and 0 kg N ha^{-1} cut^{-1} and the control, respectively (table 5).

Table 5. Effect of biofertilizer and conventional nitrogen fertilizer on crude protein content, %.

Factor	Dose	Crude protein	P - value
Biofertilizer	0	$17,8^{b}$	0,0114
	600	$18,5^{ab}$	
	1 200	$19,2^{a}$	
Nitrogen fertilizer	0	$17,2^{c}$	0,0001
	10	$18,4^{b}$	
	20	19,9	
Interaction			
Biofertilizer	Nitrogen fertilizer	Crude protein	P - value
1 200	20	$21,6^{a}$	0,0037
600	10	$19,7^{ab}$	
600	20	$19,1^{bc}$	
0	20	$19,0^{bc}$	
1 200	0	$18,2^{bcd}$	
0	10	$17,9^{bcd}$	
1 200	10	$17,8^{bcd}$	
600	0	$16,9^{cd}$	
0	0	$16,5^{d}$	
Mean		18,4	
VC, %		8,8	

Different letters in the same column indicate significant differences between treatments ($p < 0,05$).

VC: Variation coefficient

Lorentz *et al.* (2020) proved that biological fertilization improves N concentration in pastures. Also Lopes *et al.* (2020) report that biological fertilizers improve chlorophyll concentration, photosynthetic rate and water use efficiency, as well as total carbohydrate and protein content in pastures. These biological fertilizers have a synergistic effect on N fixation and P release in the soil and improve forage quality (Oberoi *et al.*, 2020). Vishnu *et al.* (2022) claim that they enhance biomass production and protein concentration in pastures.

In this research, mixtures of grasses and legumes were used. According to Bergqvist (2021), the increase in protein in the mixture is due to the contribution of the legume. There were changes in protein content in a mixture of *L. perenne* and *T. pratense*, which was due to the modifications that *T. pratense* has in the mixture (Weller and Cooper, 2008). The proportion of legumes in a mixture of *L. perenne* and *T. pratense* is related to the CP content it can achieve (Bakhtiyari *et al.*, 2020). The implementation of biofertilizers as a management strategy in fertilization provides microelements, such as boron and molybdenum, which are important for legumes, as they enhance their growth (Churkova, 2019).

Normalized difference vegetation index. In the variance analysis of NDVI, no statistical differences ($p < 0,05$) were observed between treatments for days 1 and 7 post-cutting. However, on day 7, BF and NF applications were made in each of the treatments. Therefore, the results of the applications on NDVI are shown from day 14 post-cutting, when statistical differences were observed for NF. Doses 10 and 20 kg N ha^{-1} cut^{-1} were those with the highest NDVI values (0,83 and 0,84 in the same order) compared with 0 kg N ha^{-1} cut^{-1}, which had the lowest value (0,8). No statistical differences were found for BF or for the interaction (table 6).

At day 21 post-cutting, statistical differences ($p < 0,05$) were recorded for BF, NF and the BF x NF interaction. The highest NDVI value (0,87) was obtained when 1 200 L ha^{-1} cut^{-1} were applied compared with the control, which indicated the

Table 6. Effect of biofertilizer and nitrogen fertilizer treatments on NDVI as a function of days post-cutting.

	Dose	0		7		14		21		28	
		NDVI	P - value	NDVI	P - value	NDVI	Valor - P	NDVI	P - value	NDVI	P - value
BF	0	0,71		0,74		0,81		0,85^{b}	0,046	0,86^{b}	
	600	0,72	0,72	0,77	0,06	0,83	0,067	0,86^{ab}		0,87^{a}	0,0003
	1200	0,72		0,77		0,83		0,87^{a}		0,88^{a}	
NF	0	0,71		0,75	0,25	0,80^{b}		0,84^{b}		0,85^{b}	
	10	0,72	0,32	0,77		0,83^{a}	0,004	0,87^{a}	0,0001	0,88^{a}	0,0001
Mean	20	0,7		0,75		0,84^{a}		0,87^{a}		0,88^{a}	
BF	NF	NDVI	P - value	NDVI	P - value	NDVI	P - value	NDVI	P - value	NDVI	P - value
1200	20	0,7		0,77		0,84		0,88^{a}		0,89^{a}	
600	20	0,71		0,77		0,84		0,88^{a}		0,88^{a}	
1200	10	0,72		0,77		0,84		0,86^{a}		0,88^{a}	
600	10	0,73		0,78		0,83		0,87^{ab}		0,88^{a}	
0	20	0,69	0,86	0,73	0,79	0,82	0,12	0,86^{ab}	0,0004	0,86^{a}	0,0001
0	10	0,72		0,76		0,85		0,88^{a}		0,86^{a}	
1200	0	0,73		0,77		0,82		0,86^{ab}		0,88^{a}	
600	0	0,71		0,77		0,81		0,84^{bc}		0,86^{a}	
0	0	0,7		0,72		0,77		0,82^{bc}		0,82b	
Mean		0,71		0,75		0,82		0,86		0,86	
VC, %		9,04		7,87		5,21		2,63		2,53	

NDVI: normalized difference vegetation index, BF: biofertilizer, NF: nitrogen fertilization, VC: variation coefficient.
Different letters in the same column indicate significant differences among fertilization treatments ($p < 0{,}05$).

lowest value (0,85). The highest NDVI value (0,87) was observed with the doses of 10 and 20 kg N ha^{-1} cut^{-1} and the control (0,84) was in the lowest range. In the BF x NF interaction, three ranges of significance were obtained. The highest NDVI values (0,88) were achieved with the combined applications of the two factors under study. On day 28 post-cutting, statistical differences ($p < 0{,}05$) were observed for BF, NF and the BF x NF interaction. However, it is important to note that NDVI values did not exceed those recorded prior to day 27 post-cutting. The highest NDVI values (0,88 and 0,87) were observed when 600 or 1 200 L ha^{-1} cut^{-1} were applied in the same order compared with the control. High NDVI values (0,88) were associated with the doses of 10 and 20 kg N ha^{-1} cut^{-1} versus the dose of 0 kg N ha^{-1} cut^{-1} with NDVI of 0,85. In the BF x NF interaction, no statistical differences were observed among treatments except for the control.

NDVI has a close relationship with crop yield (Guan *et al*., 2019) and is a tool for determining nutritional status and nitrogen fertilization (Edalat *et al*., 2019). NDVI generally shows the health status of a plant (Mahajan and Bundel, 2016). Sharma and Bali (2018) noted that NDVI can be used for the assessment of plant growth and leaf color, because it only analyzes green leaves and deprecates dead leaves.

In general, "healthy" crops absorb most of the radiation in the red spectrum, while they reflect most of the near-infrared radiation and as a result NDVI values are close to 1 (Pino, 2019). In this research, acceptable values were observed from day 21 post-cutting, when values of 0,86 were recorded. In this regard, Gutiérrez-Soto *et al*. (2011) mention that values between 0,7 and 0,8 are indicators of plants that are in the best conditions. On the other hand, NDVI values higher than 0,8 represent the maximum yield potential of a crop (Grohs *et al*., 2009).

Serrano *et al*. (2018) proved that NDVI detects high levels of chlorophyll (photosynthetically active vegetation), which is abundant in green vegetation, and decreases as the pasture matures and leaf senescence begins. Likewise, this index is particularly sensitive to N variations at leaf level (Vergara-Díaz *et al*., 2016) and to variations in canopy architecture (Gitelson *et al*., 2002).

Conclusions

The best biomass, crude protein and normalized vegetation index results were obtained when nitrogen fertilizers and biofertilizers were used in combination, with the highest biomass yield (3 177 kg DM ha^{-1}) associated with the 600 L and 20 kg N ha^{-1} cut^{-1} treatment and the highest crude protein (21,6 %) and NDVI (0,89) values with the 1 200 L and 20 kg N ha^{-1} cut^{-1} treatment.

Biofertilizers have low concentration of nutrients, but they have great potential, since they improve the assimilation of nitrogen fertilizers and good yields and pasture quality are achieved with relatively low doses of nitrogen between cuts.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

- Francisco Adolfo Gutiérrez-León. Field data recording with Greenseeker sensors for NDVI, dry matter determination, management of plots and biofertilizer ponds, statistical analysis and manuscript writing.
- Soraya Patricia Alvarado-Ochoa. Soil analysis and manuscript writing.
- Jaime Fabrizzio Reascos-Castillo. Plot management and sampling.
- Evelyn Nicole Ortiz-Flores. Pool management and sampling.
- Arnulfo Rigoberto Portilla-Narvaez. Protein and biofertilizer analysis.
- Marco Antonio Rivera-Montesdeoca. Soil biofertilizer analysis.

Bibliographic references

Bakhtiyari, F.; Zamanian, M. & Golzardi, F. Effect of mixed intercropping of clover on forage yield and quality. *South-West. J. Hortic. Biol. Environ.* 11 (1):e20105. https://biozoojournals.ro/swjhbe/v11n1/swjhbe_e20105_Farzaneh.pdf, 2020.

Barbieri, P. A.; Echeverría, H. E.; Saínz-Rozas, H. R. & Maringolo, M. Fertilización de maíz con urea de liberación lenta: pérdida por volatilización y eficiencia de uso de nitrógeno. *Ci. Suelo.* 28 (1):57-66. http://www.scielo.org.ar/scielo.php?script=sci_arttext&pid=S1850-20672010000100007, 2010.

Bergqvist, Sanna. Long-term effects of cropping system on red clover proportion and crude protein concentration in mixed leys. Uppsala, Sweden: Department of Crop Production Ecology, Swedish University of Agricultural Sciences. https://stud.epsilon.slu.se/17423/3/bergqvist_s_211208.pdf, 2021.

Berone, G. *Ecofisiologia aplicada al manejo de pasturas*. Argentina: INTA, 2016.

Castro., E.; Mojica, J. E.; León, J.; Pabón, Martha; Carulla, J. & Cárdenas, E. Balance de nitrógeno en pastura de gramíneas y pastura de gramínea más *Lotus uliginosus* en la sabana de Bogotá, Colombia. *Corpoica Cienc. Tecnol. Agropecuaria.* 10 (1):91-101. http://www.redalyc.org/articulo.oa?id=449945026009, 2009.

Churkova, Boryana. Impact of humate biofertilizers on the basic chemical composition of grass biomass of bird's-foot-trefoil, red clover and sainfoin. *Trakia J. Sci.* 17 (4):363-370, 2019. DOI: https://doi.org/10.15547/tjs.2019.04.012.

Edalat, M.; Naderi, R. & Egan, T. P. Corn nitrogen management using NDVI and SPAD sensor-based data under conventional *vs*. reduced tillage systems. *J. Plant Nutr.* 42 (18):2310-2322, 2019. DOI: https://doi.org/10.1080/01904167.2019.1648686.

Escobar, María I.; Navas-Panadero, A.; Medina, Claudia A.; Corrales-Álvarez, J. D.; Tenjo, Ana I. & Borrás-Sandoval, L. M. Efecto de prácticas agroecológicas sobre características del suelo en un sistema de lechería especializada del trópico alto colombiano. *LRRD.* 32 (4). http://www.lrrd.org/lrrd32/4/maria.es32058.html, 2020.

Etesami, H. Enhanced phosphorus fertilizer use efficiency with microorganisms. In: R. S. Meena, ed. *Nutrient dynamics for sustainable crop production*. Singapore: Springer. p. 215-245, 2020. DOI: https://doi.org/10.1007/978-981-13-8660-2_8.

Furey, G. N. & Tilman, D. Plant biodiversity and the regeneration of soil fertility. *PNAS.* 118 (49):e2111321118, 2021. DOI: https://doi.org/10.1073/pnas.2111321118.

Gitelson, A. A.; Kaufman, Y. J.; Stark, R. & Rundquist, D. Novel algorithms for remote estimation of vegetation fraction. *Remote Sens. Environ.* 80 (1):76-87, 2002. DOI: https://doi.org/10.1016/S0034-4257(01)00289-9.

Grohs, D. S.; Bredemeier, C.; Mundstock, C. M. & Poletto, Naracelis. Modelo para estimativa do potencial produtivo em trigo e cevada por meio do sensor Greenseeker. *Eng. Agríc.* 29 (1):101-112, 2009. DOI: https://doi.org/10.1590/S0100-69162009000100011.

Guan, S.; Fukami, K.; Matsunaka, H.; Okami, M.; Tanaka, R.; Nakano, H. *et al.* Assessing correlation of high-resolution NDVI with fertilizer application level and yield of rice and wheat crops using small UAVs. *Remote. Sens.* 11 (2):112, 2019. DOI: https://doi.org/10.3390/rs11020112.

Gutiérrez-Soto, M. V.; Cadet-Piedra, E.; Rodríguez-Montero, W. & Araya-Alfaro, J. M. El GreenSeeker™ y el diagnóstico del estado de salud de los cultivos. *Agron. Mesoam.* 22 (2):397-403. http://www.scielo.sa.cr/scielo.php?script=sci_arttext&pid=S1659-13212011000200016&lng=en&tlng=es, 2011.

Hall, Mary B. Determination of starch, including maltooligosaccharides, in animal feeds: comparison of methods and a method recommended for AOAC collaborative study. *J. AOAC Int.* 92 (1):42-49, 2009. DOI: https://doi.org/10.1093/jaoac/92.1.42.

Hoffman, E. M.; Fassana, N. C.; Mazzilli, S.; Berger, A. & Ernst, O. La necesidad de incrementar la eficiencia de uso del nitrógeno en cereales de invierno. *Fertilizar.* 24:3-7. https://fertilizar.org.ar/wp-content/uploads/2016/12/2.pdf, 2016.

INAMHI. *Características edafoclimáticas*. Quito: Instituto Nacional de Meteorología e Hidrología. https://www.inamhi.gob.ec/, 2022.

Insua, J.; Utsumi, S. Nuevas tecnologías para el monitoreo de pasturas. *Visión Rural.* https://ri.conicet.gov.ar/handle/11336/80888?show=full, 2016.

Liu, Z.; Rong, Q.; Zhou, W. & Liang, G. Effects of inorganic and organic amendment on soil chemical properties, enzyme activities, microbial community and soil quality in yellow clayey soil. *PLoS ONE.* 12 (3):e0172767, 2017. DOI: https://doi.org/10.1371/journal.pone.0172767.

Lopes, M. J. dos S.; Dias-Filho, M. B.; Castro, T. H. dos R.; Silva, E. F. da; Rêgo, Marcela C. F. & Silva, Gisele B. da. Impacts of plant growth-promoting Rhizobacteria on tropical forage grass in Brazil. *J. Agric. Stud.* 8 (1):342-356, 2020. DOI: https://doi.org/10.5296/jas.v8i1.16077.

Lorentz, Juliana F.; Calijuri, Maria L.; Assemany, Paula P.; Alves, W. S. & Pereira, O. G. Microalgal biomass as a biofertilizer for pasture cultivation: Plant productivity and chemical composition. *J. Clean. Prod.* 276:124130-122020, 2020. DOI: https://dx.doi.org/10.1016/j.jclepro.2020.124130.

Mahajan, U. & Bundel, B. R. Drones for normalized difference vegetation index (NDVI), to estimate crop health for precision agriculture: A cheaper alternative for spatial satellite sensors. G. C. Mishra, ed. *Proceedings International Conference on Innovative Research in Agriculture, Food Science, Forestry, Horticulture, Aquaculture, Animal Sciences, Biodiversity, Ecological Sciences and Climate Change.* Krishi Sanskriti Publications, New Delhi. p. 38-41. https://www.researchgate.net/publication/312497233, 2016.

Maurya, Swati; Jeeva S, Abraham; Somasundaram, Sripoorna; Toteja, R.; Gupta, Renu & Makhija, Seema. Indicators for assessment of soil quality: a mini-review. *Environ. Monit. Assess.* 28 (9):604, 2020. DOI: https://doi.org/10.1007/s10661-020-08556-z.

Nair, K. P. Soil fertility and nutrient management. In: K. P. Nair, ed. *Intelligent soil management for sustainable agriculture: The nutrient buffer power concept.* Cham, Germany: Springer International Publishing. p. 165-189, 2019. DOI: https://doi.org/10.1007/978-3-030-15530-8_17.

Neves, T. I.; Uyeda, C. A.; Carvalho, Monica & Abrahão, R. Environmental evaluation of the life cycle of elephant grass fertilization—*Cenchrus purpureus* (Schumach.) Morrone—using chemical fertilization and biosolids. *Environ. Monit. Assess.* 190 (1):30, 2017. DOI: https://doi.org/10.1007/s10661-017-6406-4.

Oberoi, H. K.; Pandove, Gulab & Kaur, Anureet. Effect of pre-sowing seed inoculation with liquid biofertilizers on fodder yield and quality of sorghum (*Sorghum bicolor*). *Indian J. Agron.* 65 (1):100-106. https://indianjournals.com/ijor.aspx?target=ijor:ija&volume=65&issue=1&article=015, 2020.

Odoh, C. K.; Sam, K.; Zabbey, N.; Eze, C. N.; Nwankwegu, A. S.; Laku, Charity *et al.* Microbial consortium as biofertilizers for crops growing under the extreme habitats. In: A. N. Yadav, J. Singh, A. A. Rastegari and N. Yadav, N., eds. *Plant microbiomes for sustainable agriculture*. Cham, Germany: Springer International Publishing. p. 381-424, 2020. DOI: https://doi.org/10.1007/978-3-030-38453-1_13.

Orozco-Corral, A. L.; Valverde-Flores, Martha I.; Martínez-Téllez, R.; Chávez-Bustillos, C. & Benavides-Hernández, R. Propiedades físicas, químicas y biológicas de un suelo con biofertilización cultivado con manzano. *Terra Latinoam.* 34 (4):441-456. http://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0187-57792016000400441&lng=es&tlng=, 2016.

Panpatte, D. G. & Jhala, Y. K., Eds. *Soil fertility management for sustainable development*. Singapore: Springer, 2019. DOI: https://doi.org/10.1007/978-981-13-5904-0.

Pino., E. Drones a tool for efficient agriculture: a high-tech future. *Idesia*. 37 (1):75-84, 2019. DOI: https://dx.doi.org/10.4067/S0718-34292019005000402.

Saharan, B. S. & Nehra, V. Plant growth promoting rhizobacteria: a critical review. *Life Sci. Med. Res.* 21:LSMR-21. https://www.researchgate.net/publication/284340739, 2011.

Serrano, J.; Shahidian, S. & Silva, J. M. da. Monitoring seasonal pasture quality degradation in the Mediterranean montado ecosystem: Proximal versus remote sensing. *Water.* 10:1422, 2018. DOI: https://doi.org/10.3390/w10101422.

Sharma, L. K. & Bali, S. K. A review of methods to improve nitrogen use efficiency in agriculture. *Sustainability.* 10:51, 2018. DOI: https://doi.org/10.3390/su10010051.

Silveira, Maria L. & Kohmann, Marta M. Maintaining soil fertility and health for sustainable pastures. In: Monte Rouquette and Glen E. Aiken, eds. *Management strategies for sustainable cattle production in southern pastures*. USA: Academic Press. p. 35-58, 2020. DOI: https://doi.org/10.1016/B978-0-12-814474-9.00003-7.

Sofo, A.; Mininni, Alba N. & Ricciut, Patrizia. Soil macrofauna: A key factor for increasing soil fertility and promoting sustainable soil use in fruit orchard agrosystems. *Agronomy.* 10:456, 2020. DOI: https://doi.org/10.3390/agronomy10040456.

Thiex, Nancy J.; H. Manson, Shirley Andersson & Persson, J.-A. Determination of crude protein in animal feed, forage, grain, and oilseeds by using block digestion with a copper catalyst and steam distillation into boric acid: collaborative study. *J. AOAC Int.* 85 (2):309-317. https://pubmed.ncbi.nlm.nih.gov/11990013/, 2002.

Tilman, D.; Wedin, D. & Knops, J. Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature.* 379 (6567):718-720, 1996. DOI: https://doi.org/10.1038/379718a0.

Trotter, M. G.; Lamb, D. W.; Donald, G. E. & Schneider, D. A. Evaluating an active optical sensor for quantifying and mapping green herbage mass and growth in a perennial grass pasture. *Crop Pasture Sci.* 61 (5):389-398, 2010. DOI: https://doi.org/10.1071/CP10019.

Vergara-Díaz, O.; Zaman-Allah, M. A.; Masuka, B.; Hornero, A.; Zarco-Tejada, P.; Prasanna, B. M. *et al.* A novel remote sensing approach for prediction of maize yield under different conditions of nitrogen fertilization. *Front. Plant Sci.* 7:666, 2016. DOI: https://doi.org/10.3389/fpls.2016.00666.

Vishnu, P.; Yasasvi, B. & Tarate, S. B. Influence of biofertilizers on millet production. *The Pharma Innovation Journal.* 11 (2):950-953. https://www.thepharmajournal.com/archives/?year=2022&vol=11&issue=2&ArticleId=10656, 2022.

Wallace, B. M.; Krzic, Maja; Forge, T. A; Broersma, K. & Newman, R. F. Biosolids increase soil aggregation and protection of soil carbon five years after application on a crested wheatgrass pasture. *J. Environ. Qual.* 38 (1):291-298, 2009. DOI: https://doi.org/10.2134/jeq2007.0608.

Walsh, Olga S.; Klatt, A. R.; Solie, J. B.; Godsey, C. B. & Raun, W. R. Use of soil moisture data for refined GreenSeeker sensor based nitrogen recommendations in winter wheat (*Triticum aestivum* L.). *Precis. Agric.* 14 (3):343-356, 2013. DOI: https://doi.org/10.1007/s11119-012-9299-9.

Weller, R. F. & Cooper, A. Seasonal changes in the crude protein concentration of mixed swards of white clover/perennial ryegrass grown without fertilizer N in an organic farming system in the United Kingdom. *Grass Forage Sci.* 56 (1):92–95, 2008. DOI: https://doi.org/10.1046/j.1365-2494.2001.00248.x.

Wiesmeier, M.; Urbanski, Livia; Hobley, Eleanor; Lang, Birgit; Lützow, Margit von; Marin-Spiotta, Erika *et al.* Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. *Geoderma.* 333:149-162, 2019. DOI: https://doi.org/10.1016/j.geoderma.2018.07.026.

Wigley, Kathryn; Owens, Jennifer L.; Westerschulte, M.; Riding, P.; Fourie, J. & Werner, A. Photogrammetry for assessment of pasture biomass. *J. N. Z. Grassl.* 81:33-40, 2019. DOI: https://doi.org/10.33584/jnzg.2019.81.394.