Hindawi Publishing Corporation
Applied and Environmental Soil Science
Volume 2011, Article ID 847940, 10 pages
doi:10.1155/2011/847940

Research Article

Biological and Physicochemical Parameters Related to the Nitrogen Cycle in the Rhizospheric Soil of Native Potato (*Solanum phureja*) Crops of Colombia

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Received 24 November 2010; Accepted 19 May 2011

Academic Editor: Paul Voroney

Nitrogen (N) plays an important role in agricultural production. This study was designed to evaluate the presence of cultivable N cycle-associated microorganisms (nitrogen-fixing bacteria—NFB, proteolytic bacteria—PR, ammonifiers—AMO, ammonium-oxidizing bacteria—AOB, nitrite-oxidizing bacteria—NOB, and denitrifiers—DEN), and their relationship with physical-chemical and agronomic soil descriptors, in *Solanum phureja* rhizospheric soil samples, from traditional and organic crop management farms. A cluster analysis with the physical and chemical properties of soil, allowed to identify the organic matter content as an important factor that determines the outcome of that grouping. Significant differences ($P < 0.05$) between farms were found in the abundance of this groups, but correlation analysis showed that proteolytic and nitrogen fixing bacteria were the main nitrogen associated functional groups affected by soils' physical-chemical characteristics. The amount of ammonia available is affected by the agricultural management strategy, which consequently affects the NFB abundance. Finally the results showed that PR, protease activity and soil properties related with organic matter transformation has a positive relationship with productivity, which given the high organic matter content of the Andean soils being studied, we conclude that nitrogen mineralization process has an important role in the nitrogen cycle and its bioavailability in this ecosystem.

1. Introduction

Potato is the world's fourth most popular food crop and one of the most important agricultural products for Colombia; it occupies the ninth place in area sown regarding transitory crops and sixth place in production value [1]. *Solanum phureja* is a native potato representing around 10% of polinebreak tato crops produced in Colombia (having around 25,000 hectares planted and 150,000 tons produced per year), making Colombia the highest producer of such native potato amongst Andean countries [2–4].

The regions where potato crops are planted in Colombia are characterized by having a cold to very cold climate, due to their high altitude in the Andean mountains (2,000 meters above sea level (masl) to 3,500 masl), where fragile ecosystems such as Paramo are located. Such climatic conditions define important soil characteristics since they limit organic matter's mineralization speed, this being one of the reasons for which the aforementioned soils are characterized by presenting high organic matter content (usually above 10%). The volcanic ash from andisols is considered to be the material of origin partially or completely affecting these soils' fertility, because their organic and mineral composition is dominated by allophane and imogolite in their clay fraction, thereby interfering with nitrogen mineralization [5]. Besides, the humus-aluminum complex (a fraction acting as high immobilizer of available phosphorus in soil solution) also affects soil fertility [6].

The limiting condition of nutrients in soils is vitally important for potato growing because it is well know that such crops need to extract high quantities of nutrients from soil. This explains the favorable response to fertilization, given that major yields result from high nutrient input [7]. Nevertheless, potato farmers' increased fertilizer use has become

much greater than the productivity obtained [8]. Nowadays potato growers occupy second place in chemical fertilizer consumption in Colombia (following coffee producers), representing 14% to 23% of entire potato production costs [9], so fertilization thus appears to be a critical factor determining sector competitiveness (without emphasizing its environmental impact).

Potato is a very sensitive crop to nitrogen fertilization, especially in soils having high organic matter where such fertilization may reduce tuber yield and quality [10]. Excess nitrogen may prolong the vegetative phase and therefore interfere with the initiation of tuberization, decreasing yield and the percentage of dry matter accumulation in the tubers [11]. On the other hand, a low nitrogen application rate may produce premature senescence in the plants due to early translocation of nitrogen from the leaves to the tubers [12]. The low efficiency of species such as *S. tuberosum* in assimilating nitrogen has been well documented [13, 14]; an inappropriate use of this fertilizer may thus create environmental problems. For instance, unbalanced fertilization may intensify nitrogen leaching into water sources as well as N gaseous emission [15–17]. Forms of reactive nitrogen input to ecosystems as a consequence of anthropogenic activities, such as agriculture and industry, should be expected to have implications for soil microorganisms mediating such transformation, as well as the cycle's global dynamics, since it has been estimated that around 95% of nitrogen flow in the global terrestrial system is restricted to the plant-microorganism-soil system [18]. It has thus been estimated that around 50% of the nitrogen applied as mineral fertilizer has a negative effect on the environmental metabolism of nitrogen, thereby supporting the environmental quality of N [19].

Accordingly, ways must be sought for making fertilization practice more efficient, for example by studying the microbial communities involved in nutrient biotransformation such as nitrogen because such transformation determines its bioavailability for the plant. Taking this into account, our objective was to evaluate the abundance of some functional groups of N-cycling microorganisms (biological nitrogen fixers, proteolytic bacteria, ammonifiers, ammonium and nitrite oxidizers, and denitrifiers) and its relationship with other biochemical and physical-chemical characteristics, associated with the edaphic metabolism of nitrogen in the native potato (*Solanum phureja*) crops. This study was conducted under the hypothesis that soil physical-chemical properties can affect the population size of such functional groups as well as soil' enzymatic activity, and the establishment of specific relations allows us to identify important points of N cycle that regulates its bioavailability under *S. phureja* crop conditions.

2. Materials and Methods

2.1. Soil Samples. Rhizospheric soil samples were collected from seven *S. phureja* crops located in the Cundinamarca department, one of the major native potato sowing areas in Colombia. Five of these crops were being traditionally managed with 700 to 900 kg ha^{-1} of 12 : 24 : 12 or 14 : 30 : 15 NPK fertilizer application rates. Another two crops were being organically managed; they were characterized by compost amendments being applied for fertilization. The crop area was sampled by taking five rhizospheric soil samples for each crop covered in zigzag, leaving a 20–25 meter distance between sampled plants. Each of the five subsamples was sifted by 0.5 cm diameter sieve and then mixed in equal parts to obtain two compound samples per farm one stored at 4°C for microbial analysis and the other at −20°C for enzymatic analysis. Additional data regarding productivity and fertilization plan were analyzed for traditionally managed farms.

Physical-chemical analysis of soils was done in the Laboratory of Soils of the Agronomy College at the Universidad Nacional de Colombia, using standard methodologies for the measurement of moisture, pH, organic carbon (OC), Ca, K, Mg, Na, cationic exchange capacity (CEC), P, Cu, Fe, Mn, clay, silt and sand percentage, total nitrogen (inorganic plus organic), ammonium, and nitrate.

2.2. Microbial Count of Edaphic Nitrogen Metabolism-Associated Functional Groups. Nitrogen-fixing bacteria were analyzed by plate count in modified NFB agar [20], (components per liter: 5 g malic acid, 0.5 g K_2HPO_4, 0.1 g NaCl, 0.2 g $MgSO_4 \cdot 7H_2O$, 0.02 g $CaCl_2 \cdot 2H_2O$, 0.05 g Bromothymol Blue, 2 g KOH, 8 g mannitol, 8 g glucose) supplemented with 2 mL micronutrient solution (components per liter: 0.2 g $Na_2MoO_4 \cdot 2H_2O$, 0.235 g $MnSO_4$, 0.280 g H_3BO_3, 0.08 g $CuSO_4 \cdot 5H_2O$, 0.0024 g $ZnSO_4 \cdot 7H_2O$), 1 mL of vitamin solution 0.1 g L^{-1} (biotin, 0.2 g L^{-1}pyridoxine) and 4 mL of Fe-EDTA solution (5.56 g $FeSO_4$ 7 H_2O L^{-1}, 8.258 g L^{-1} Na_2 EDTA) and solidified with 1.5% Oxoid agar-agar, pH was fixed at 7.0. Each plate was incubated for 5 days at 28°C; all soil samples were analyzed in triplicate.

The abundance of proteolytic microorganisms (PR), ammonifiers (AMO), ammonium oxidizers (AOB), nitrite oxidizers (NOB), and denitrifiers (DEN) was measured by the most probable number (MPN) method. Briefly, culture media was prepared using Winogradsky's salt solution diluted to 50 mL·L^{-1} and supplemented with a 1 mL·L^{-1} microelement solution [21]. Different carbon and nitrogen sources were used according to the group of interest. Gelatine (30 g·L^{-1}) was used for PR as the sole carbon and nitrogen source, whereas asparagine (0.2 g L^{-1}) was used for AMO. Calcium carbonate (1 g L^{-1}) was used as carbon source and ammonium sulphate (0.5 g L^{-1}) and sodium nitrite (5 g L^{-1}) as nitrogen source for BOA and BON. DEN were grown with potassium nitrate (2 g L^{-1}) and sodium acetate (10 g L^{-1}). Tubes were incubated in triplicate for 15 days at 30°C for PR, AMO, and DEN, and 30 days for BOA and BON. Each microbial population was estimated in specific growth media depending on the test. Gelatin liquefaction was used for determining PR abundance [22]. Ammonium production was used for determining AMO by Nessler's reagent [23]. Nitrite production for BOA estimation and nitrite consumption for BON estimation were determined with Griess Ilosvay reagent [24]. Nitrite consumption and gas production were determined by Durham bell for estimating DEN [25]. Microorganism MPN was calculated by using

McCrady tables [26]; counts were expressed as microorganism MPN by gram of dry soil.

2.3. Determining Protease and Nitrogenase Activity in Soil Samples. Protease activity was measured as suggested by Ladd and Butler [27]; briefly 0.1 g of soil was incubated for 2 hr at 50°C in 1 mL of casein (2% solution) as substrate in alkaline conditions (Tris buffer pH 8.1). Amino acids released during incubation were colorimetrically measured at 700 nm using Folin-Ciocalteu reagent. A standard curve was constructed with tyrosine to find the concentration of amino acids released by the sample's enzymatic activity. Each sample was evaluated five times; two reaction controls were used (samples incubated without substratum). Soil nitrogenase was estimated by the acetylene reduction activity (ARA) test [28]. One mL from different soil sample dilutions was incubated in triplicate in bottles containing 9 mL semisolid NFB Agar. Inoculated bottles were incubated for 24 hours at 30°C; 10% of the bottle's gaseous space was then replaced by acetylene, and then each sample was incubated again for 24 hours. Once incubation time had elapsed, the amount of produced ethylene was determined on a Varian 3400 gas chromatograph (Varian, Walnut Creek, Calif, USA). Nitrogenase activity was measured in triplicate for each farm and the results expressed in n moles of ethylene per gram of dry soil in 24 hours.

2.4. Statistical Analysis. Microbial count analysis was expressed in logarithmic form, and thus an ANOVA was done for the abundance of each group. However, since data did not support normality assumption, we used a nonparametric approach to verify statistically significant differences through the Kruskal-Wallis (KW) test by pairs at $P = 0.05$ significance level [29]. Enzymatic activity differences were analyzed by Tukey's test at the same significance level. Correlation between the study variables was also analyzed using STATA 9.0. [30]. Relationships among the observed variables were analyzed by principal component analysis (PCA) using the *R* statistical package [31]. A hierarchical cluster analysis was done using each soil's physical-chemical and biological variables, according to average linkage clustering method in STATA 9.0. [30].

3. Results

Table 1 presents the results of the physical-chemical analysis of the soil samples being studied; differences between farms were found independently of agricultural management, especially in organic carbon content, total nitrogen, soil moisture percentage, and soil texture patterns ranging from clay to clay loam (according to clay, silt, and sand percentage). pH values were observed from moderately acid to strongly acid, organic farms having the highest pH values. It should be noted that farms having organic management presented the lowest ammonium concentrations compared to traditional farms, except farm 1 which exhibited the highest total (organic) nitrogen and calcium content. Likewise, distinguishing characteristics between organically and traditionally managed farms were sodium (higher in organically managed farms) and iron concentration (higher in traditionally managed farms).

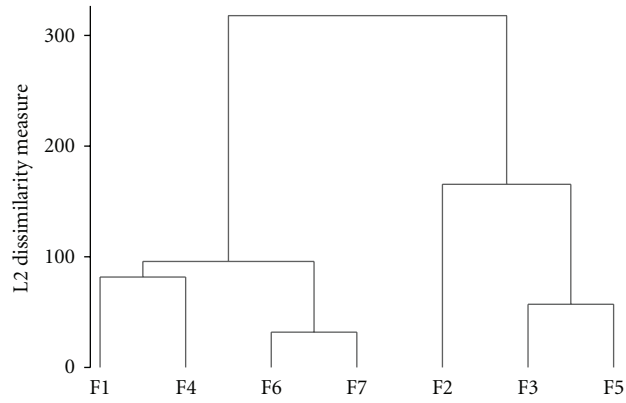

Figure 1: Hierarchical cluster analysis of the studied soils in relation to the physical-chemical variables evaluated.

It was observed that the organic matter content of soils appeared to be a determinant factor in the observed clusters when performing hierarchical cluster analysis with the results of the physical-chemical variables evaluated (Figure 1). Farms having higher organic matter content (farms 1, 4, 6, and 7) were grouped in one cluster in this analysis and were separated from farms having lower organic matter content (2, 3 and 5). This result suggested that organic matter may have an important role in the physical-chemical differences of the soils being studied. It appeared that joint analysis of the physical-chemical parameters grouped the farms according to agricultural management, since it was observed how the organically managed farms (6 and 7) were grouped and separated from traditionally managed farms (1 and 4) within the cluster of fields having high organic matter content (1, 4, 6 and 7).

Figure 2 presents the results of the nitrogen-fixing bacteria (NFB) counts. The highest count was for traditionally managed farms 3, 4, and 5 followed by farm 2 which presented intermediate values. Traditionally managed farm 1 and organically managed farms 6 and 7 presented the lowest counts. The relationship between NFB count and physical-chemical characteristics of the soil showed a positive correlation ($P < 0.05$) with ammonium concentration (r^2 : 0.8650), whereas there was a negative correlation with sand (r^2 : −0.9174) and sodium concentration (r^2 : −0.9361). There was positive correlation ($P < 0.10$) with silt (r^2 : 0.7178), clay (r^2 : 0.7605) and iron concentration (r^2 : 0.6858) and negative correlation with pH (r^2 : −0.7363) and moisture (r^2 : −0.7006).

Table 2 gives the microbial count for other groups associated with the edaphic metabolism of nitrogen. The results indicated a significant difference between farms for all microbial groups estimated, except for proteolytic microbial counts which did not present statistical differences between farms. DEN bacteria presented the lowest count in organically managed farm 6; intermediate values were found in

Table 1: Physical-chemical analysis of the soils being studied.

Farm	Agricultural management	Production	% H	pH	OC	Ca	K	Mg	Na	CEC	P	Cu	Fe	Mn	Zn	Clay	Silt	Sand	N	NH_4	NO_3
F1	T[1]	23.38	79.10	5.6	14.5	11.1	1.44	1.67	0.28	69.3	11.7	1.43	170	3.01	3.57	10	39	51	1.08	24.5	43
F2	T[1]	17.85	37.8	5.3	6.47	3.91	0.56	1.01	0.29	30.1	116	0.73	334	2.49	4.84	26	30	44	0.41	17.1	14.4
F3	T[1]	18.50	40.5	4.9	7.38	3.85	2.49	1.95	0.22	28.9	116	0.97	471	4.1	5.8	24	28	48	0.52	29.5	39.4
F4	T[1]	14.50	52.8	5.5	12.9	3.03	1.8	0.89	0.12	42.7	73.7	0.82	182	1.98	6.83	15	35	49	0.59	31.2	12.7
F5	T[1]	16.80	29.4	5.3	6.2	3.17	2.51	1.67	0.2	22.6	107	0.83	523	2.41	5.01	31	33	35	0.39	26.1	32.6
F6	O[2]	ND[3]	80	5.7	13.3	6.6	2.25	2.09	0.51	48.2	69.8	1.29	105	3.94	6.79	6	19	75	0.93	11.1	15.5
F7	O[2]	ND[3]	62.33	6.0	9.74	5.7	1.64	1.57	0.38	43.8	49.8	0.88	97	2.89	2.71	6	27	67	0.62	11.7	7.94

AM: Agricultural Management, [1]T: traditional, [2]O: organic, [3]ND: not determined; production: bundles per bundle sown, %H: percentage of humidity, OC: percentage of organic carbon, Ca: calcium (mmol·100 g^{-1}), K: potasium (mmol·100 g^{-1}), Mg: magnesium (mmol·100 g^{-1}), Na: sodium (mmol·100 g^{-1}), CEC: cation-exchange capacity (meq·100 g^{-1}), P: phosphorus (mg·Kg^{-1}), Cu: copper (mg·Kg^{-1}), Fe: iron (mg·Kg^{-1}), Mn: manganese (mg·Kg^{-1}), clay: percentage of clay, silt: percentage of silt, sand: percentage of sand, N: percentage of total nitrogen, NH_4: ammonium (mg·Kg^{-1}), and NO_3: nitrate (mg·Kg^{-1}).

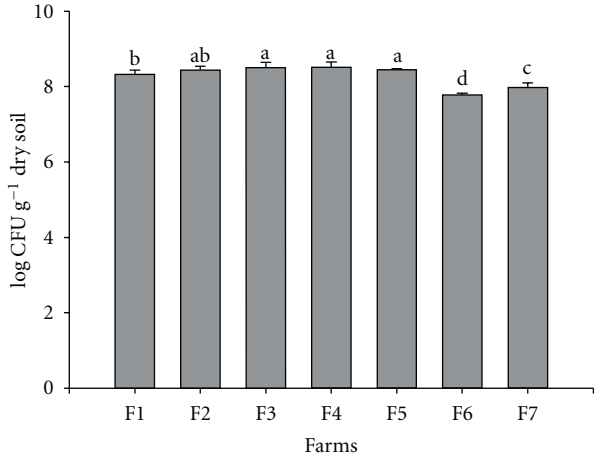

Figure 2: Nitrogen fixing bacteria count in NFB agar. Values having the same letter were not significantly different according to Kruskal Wallis pairwise test, $P = 0.05$ significance level. The error bars shown correspond to the standard deviation of the microbial count for each farm.

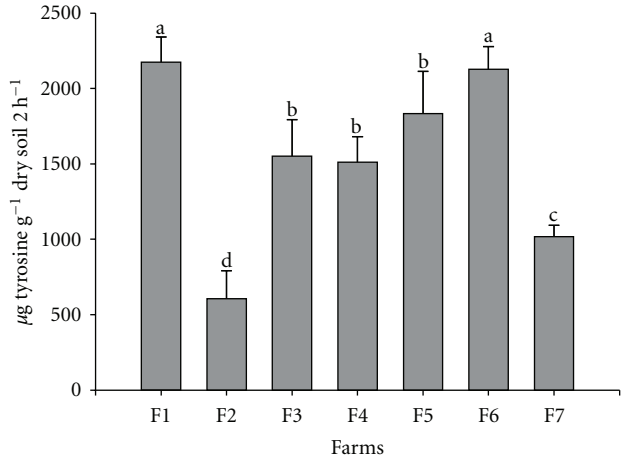

Figure 3: Protease activity. The bars correspond to average enzymatic activity, expressed in μg of tyrosine per gram of dry soil during $2\,h^{-1}$. Values shown with the same letters were not significantly different according to Tukey's test, $P = 0.05$ significance level. The error bars shown correspond to standard deviation for each data set.

farms 2, 3, and 7 followed by farms 1, 4, and 5, all of them having traditional management and presenting the highest counts. Functional groups PR and AMO were close to three orders of magnitude (2×10^7 to 4×10^8 mpn g^{-1} dry soil), this being higher than BOA and BON count (1.5×10^2 to 8×10^3 mpn g^{-1} dry soil).

Correlation analysis between the abundance of different functional groups, productivity, and other physical-chemical soil characteristics revealed a positive correlation ($P < 0.05$) between proteolytic bacteria and crop productivity (r^2 : 0.9236), soil moisture (r^2 : 0.8255), cation exchange capacity (r^2 : 0.9394), copper (r^2 : 0.8435) and total nitrogen (r^2 : 0.8992), and $P < 0.10$ for organic carbon (r^2 : 0.7491). Otherwise, this microbial group had a negative correlation

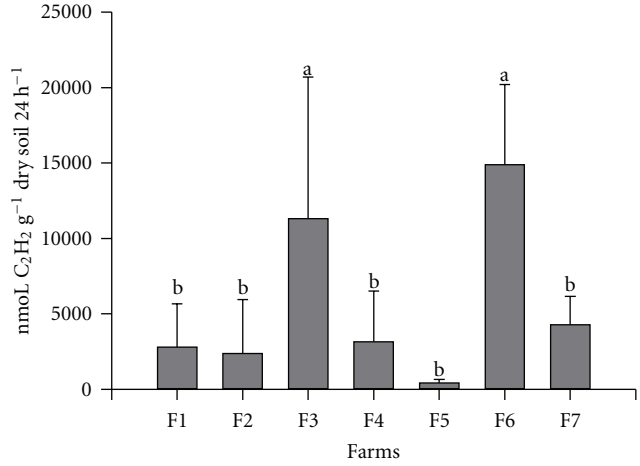

Figure 4: Nitrogenase activity. The bars correspond to average enzymatic activity, expressed in n moles of ethylene per gram of dry soil during 24 hours. Values shown with the same letter were not significantly different according to Tukey's test, $P = 0.05$ significance level. The error bars correspond to standard deviation bars for each data set.

($P < 0.05$) with phosphorus (r^2 : −0.8840). Other functional groups did not present statistically significant relationships.

The enzymatic activity results revealed statistically significant differences between farms. For instance, farms 1 and 6 had the highest protease activity (traditionally and organically managed, resp.) (Figure 3). The soil samples from these farms also presented the highest levels of organic carbon and total nitrogen (Table 1). Protease activity had a positive correlation ($P < 0.05$) with copper (r^2 : 0.7839).

Regarding nitrogenase activity, there was no correlation between nitrogen-fixing microbial count and nitrogenase activity. For instance, the lowest NFB count was found in the soil sample from farm 6, even though nitrogenase activity was highest (Figure 4); however, nitrogenase activity and microbial count were both high in soil samples from farm 3. Such enzymatic activity had a significant correlation ($P = 0.05$) with AMO bacteria (r^2 : −0.9018), manganese (r^2 : 0.8802), and also with magnesium (r^2 : 0.6734) ($P = 0.1$).

PCA was also performed for identifying the main variables related to the nitrogen cycle in this study. Figure 5 shows the analysis explaining the variability of this set of data.

4. Discussion

N cycle is a very important process in any agricultural system but particularly in potato crops where both nitrogen excess and deficiency may interfere with the duration of the crop cycle and therefore tuber production yield [10]. The native potato *S. phureja* is planted in Colombia in high mountains soils, having high organic content and low temperature and pH values; our study showed that such characteristics are determinant for N cycling-associated microbial activity, where N mineralization by proteolytic bacteria seems to play a major role.

Table 2: Most probable number for some functional groups associated with the edaphic metabolism of nitrogen. The average of three values is shown for every functional group, expressed as mpn g^{-1} dry soil. Values having the same letter were not significantly different according to Kruskal Wallis pairwise test, $P = 0.05$ significance level.

Farms	Proteolytic	Ammonifiers	Ammonium-oxidizing bacteria	Nitrite oxidizing bacteria	Denitrifiers
F1	$4.12 \times 10^{8^a}$	$7.10 \times 10^{8^a}$	$1.15 \times 10^{2^a}$	$1.58 \times 10^{4^a}$	$> 2.51 \times 10^{6}$
F2	$2.62 \times 10^{8^a}$	$2.39 \times 10^{8^{ab}}$	$2.08 \times 10^{2^{ab}}$	$9.19 \times 10^{3^a}$	$9.28 \times 10^{5^a}$
F3	$2.81 \times 10^{8^a}$	$4.45 \times 10^{7^b}$	$9.65 \times 10^{1^{ab}}$	$> 1.97 \times 10^{4}$	$9.46 \times 10^{5^a}$
F4	2.85×108^a	$1.63 \times 10^{8^{ab}}$	$1.02 \times 10^{4^c}$	$8.90 \times 10^{4^c}$	$> 2.14 \times 10^{6}$
F5	$2.26 \times 10^{8^a}$	$1.14 \times 10^{8^{ab}}$	$5.43 \times 10^{1^{abc}}$	$7.55 \times 10^{3^{ab}}$	$> 1.81 \times 10^{6}$
F6	$2.84 \times 10^{8^a}$	$9.97 \times 10^{6^c}$	$6.99 \times 10^{2^{ab}}$	$1.79 \times 10^{3^b}$	$2.44 \times 10^{2^b}$
F7	$2.30 \times 10^{8^a}$	$1.46 \times 10^{8^{ab}}$	$2.00 \times 10^{2^b}$	$6.98 \times 10^{3^{abc}}$	$1.33 \times 10^{6^a}$

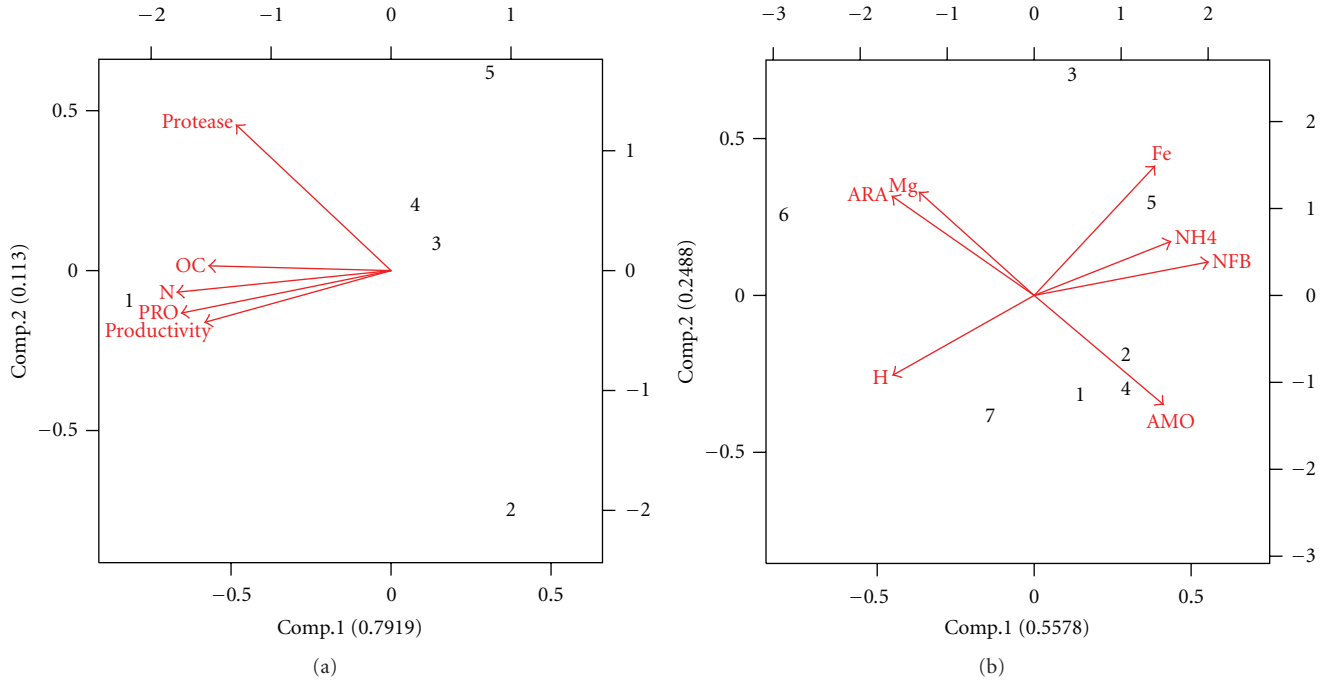

Figure 5: Principal component analysis of physical-chemical, biochemical, and biological data related to nitrogen cycle. The parameters represented in this analysis were: (a) protease: protease activity, OC: organic carbon, N: percentage of total nitrogen, PRO: proteolytic bacteria counts, productivity: crop productivity, and (b) ARA: nitrogenase activity, Mg: magnesium, H: moisture percentage, AMO: ammonifiers, NFB: nitrogen-fixing bacteria counts, NH4: ammonium, Fe: iron.

Despite these soils' high organic matter, potato growers usually apply high N fertilization input thereby affecting the microbial population and soil physical-chemical parameters. In the literature, contradictory results are found to describe the effect of agricultural practices on these physical-chemical soil variables. Some authors reported few differences in this variables in relationship with the agricultural management [32]. However, others [33, 34] observed differences in physical-chemical variables in the tested soils in relation to the type of amendment applied (organic or mineral fertilizers). For instance higher pH values [32, 33], and calcium levels [32–34], had been associated with organic management, and similar results were observed in our study (Table 1). Other physical-chemical characteristics that showed some differences between soil management strategies include the ammonium content in soils of mineral fertilized farms, possibly as a result of the incorporation of this element in the scheme of fertilization. Since in soil ammonium is fixed to clay [35], whereby a positive correlationship between these variables was expected, however we did not find such correlation in our study. On the other hand, high concentrations of sodium were found in the soils with organic management, however there is not an obvious reason to support the effect of organic amendments on the accumulation of such element [36].

As described above organic matter content of soils appeared to be a determinant factor in the observed clusters when performing hierarchical cluster analysis with the results of the physical-chemical variables evaluated. Sparling and colleagues [37] emphasized the importance of organic matter in soil because of influence on soil's physical, chemical, and biological properties; then it is possible that in the soils being studied, this was a factor that determined the physical and

biological properties of soil as well as the relations between them.

In the present study, for instance, NFB had a positive relationship with ammonium concentration in the soil. As it is well know that ammonium may inhibit nitrogenase activity [38–40], then such correlation may be the result of this group's better growth efficiency in response to available ammonium in the soil, more energy being available for cell proliferation instead of energy being wasted through the high energetic cost of nitrogen fixing as a source of ammonium [41].

A negative relationship was also found between the number of NFB and soil moisture. Such relationship was not expected since oxygen availability becomes reduced in humid environments, a factor which might benefit microorganisms depending on biological fixation for their development since it has been shown that oxygen can regulate nitrogenase activity and enzyme stability [42, 43]. However, concerning the availability of ammonium present in those soils, oxygen was probably not the limiting factor for NFB growth which included great microorganism diversity having different oxygen affinities such as aerobes, microaerophiles, facultative anaerobes, and obligate anaerobes [44].

Despite N fixation seeming to play no major role in the soils being analyzed, an expected correlation between the number of NFB and Fe was found, possibly because Fe is a nitrogenase enzyme cofactor [45]. A positive correlation was found between nitrogenase activity in soils and Mg which acts as the cofactor for ATP transport-related enzymes inside cells, this being an energetic molecule in high demand in this process [44]. This suggested that, despite the proliferation of NFB associated with the metabolism of ammonium present in the environment, NFB was an active group which might participate in the biological input of nitrogen to the soil environment in the *S. phureja* crops being studied. The positive relationship of NFB with clay and silt, which are textural characteristics influencing microorganism abundance, was anticipated since they are capable of harboring surface-adhered bacteria providing a physical protection and restricted mobility barrier ensuring these microorganisms' greater retention in the soil [46]. Despite the relationships between NFB and some physical-chemical variables, no relationship was found of this group with agronomic variables such as fertilization (kg ha^{-1} of nitrogen, potassium, or phosphorus applied) and production.

Regarding proteolytic microorganisms, a relationship was found between variables involved in organic matter mineralization such as moisture, cation-exchange capacity, organic carbon total N content (N which can be mineralized), and productivity (Figure 5). As described by Arslan et al. [47], N mineralization in the soil is a key process in N cycling, and soil characteristics such as organic matter, water holding capacity, and total N are important environmental factors affecting this soil's process. Therefore, relationships found between proteolytic microorganisms, productivity, and N mineralization-related physical chemical properties suggest that the N entry mechanism through its mineralization from organic matter sources plays an important role in the production system, despite the high levels of inorganic fertilizer applied in the fields under study (700 to 900 kg ha^{-1} of which between 90 and 150 kg ha^{-1} are nitrogen). Such influence of organic nitrogen has been previously discussed by Gastal and Lemaire [48] who suggested that although the acquisition of N by plants is generally in inorganic forms as ammonium or nitrate, soil organic N can also be used by the plant, even representing a significant portion of the total N absorbed, in particular ecological conditions such as those of acid soils and low-temperature environments, these being characteristic of the soils in our study.

Likewise, Jackson et al. [19] have argued that the N cycle in soil is driven by soil organic matter which contains approximately 5% N, as well as other labile sources of carbon and N, such as radical exudates, dead microbial cells, and cellular excretion products. According to these authors, this organic material is depolymerized by the action of extracellular enzymes releasing monomers like amino acids which, in turn, are broken down by mineralization-releasing ammonium which can be successively used for ammonium-oxidizing bacteria, nitrite oxidizers, and denitrifying bacteria, thereby ensuring the flow of N throughout the ecosystem. These authors have also highlighted that the carbon and nitrogen cycles are closely related, since microbial processes can address N conversion into forms available to plants when the right amount of carbon is available. In the same way, L. Böeme and F. Böeme [49] said that proteases activity is necessary to release N for plant uptake because most of the N compounds in mineral soils are organically bound. Therefore, if N conversion through proteolytic activity is a process which addresses this element's cycle in the soil, the enzyme-driven depolymerization should be the limiting step in generating bioavailable N. This suggests that the most appropriate way for determining organic N depolymerization would be protease activity determination [50]. In the present study, overall protease activity showed no correlation with other parameters related with the mineralization of organic matter. However, there was a general tendency concerning farms having greater amounts of organic carbon and total N, also presenting the highest protease activity, confirming the reports of other authors such as Fuka et al. [51], Kunito et al. [52], and Saha et al. [53].

On the other hand, farm 5 which presented the lowest organic carbon and total N levels also had high proteolytic activity, even being comparable to that of farms 1 and 6 therefore suggesting that other factors were affecting soil protease activity. This farm contained the highest percentage of clay which, as suggested by Fuka et al. [51], influences proteolytic activity due to protease stabilization on clay particles whereas enzymatic activity in sandy soil only depends on the activity of enzymes released by soil microorganisms. Then, excluding farm 5 from correlation analysis led to significant positive correlation of protease activity with production, organic carbon, cation-exchange capacity, moisture, and total nitrogen (not shown results). This result in addition to the high heterotrophic microbial (proteolytic and ammonifying bacteria) counts, and their relationship with soil physical-chemical parameters related to organic matter mineralization would again support that N mineralization apparently plays an important role in N cycling in *S. phureja* crops

directly interfering with their productivity. As it is known that the soil N cycle can be divided into three subcycles: elemental, autotrophic, and heterotrophic, which compete for one or more of the soil N pools, such as the ammonium pool, and the outcome of such competition determines which subcycle dominates [35], it may thus be possible that the heterotrophic subcycle (mineralization) dominates N transformation over the elemental subcycle (biological oxidation-reduction reactions) in the soils being studied which have high organic matter content.

This study found a positive relationship between protease activity and copper levels measured in the soil. Similar findings were obtained by Kunito et al. [52] who indicated that these results may have been due to multicollinearity between determined copper, soil organic carbon, and pH. The phenomenon could have been similar in our case as the copper concentration was also associated with moisture, organic carbon and total N, being variables expected to favor such enzyme activity. Moreover, while it has been recognized that metalloproteases are the main source of soil peptidases [22], to the best of our knowledge, copper has not yet been described as being a cofactor that enhances its activity.

5. Conclusions

Organic nitrogen contribution to the system through proteolytic microorganisms appeared to be important in the N cycling of *Solanum phureja* rhizospheric soils under study, which are typical Andean high mountain potato's crop soils with high organic matter content and low temperature and pH. Thus, other functional groups such as those associated with biological nitrogen fixation seemed to play a secondary role in incorporating N into the soil in this type of ecosystem. This was probably because N contribution is largely mediated by mineral fertilization or by organic sources, thereby reducing the incentive for NFB to increase edaphic N.

The other functional groups, involved in soil N metabolism in this study, showed no close relationship to the physical-chemical variables of soil and crop agronomic characteristics such as fertilization and production, which would afford no possible reason for their use as N cycle markers in ecosystems similar to that of the native *S. phureja* potato.

Acknowledgments

The authors would like to thank the Colombian Ministry of Agriculture and Rural Development and CENICAFE (the National Federation of Coffee Growers' Research Institution) which provided the financial support for this paper. We would also like to thank the members of the CIMA research consortium (Consorcio de Investigación en Metagenomica Agricola) for supporting this paper. They are grateful to Fernando Tunjano from FEDEPAPA for his help in finding the farms and the farmers who allowed them to incorporate their potato fields in this work.

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