Impact of Nitrogen Fertilization on Soil and Aquifers in the Humid Pampa, Argentina

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Abstract: There is great concern worldwide about air and water pollution arising from N-fertilizer. Considering the expansion of agriculture and fertilization practices in Argentina, the objective of this study was to determine the effect of N-fertilizers on soil N dynamics and then relationship with nitrate concentration in aquifers. Soil samples were taken during a maize crop cycle in two agricultural farms under different fertilization treatments: UAN (urea, ammonium nitrate and ammonium tiosulfate, 110 kg N ha^{-1}); and UREA (urea, 60 kg N ha^{-1}). UAN and UREA treatment produced an increase in soil nitrate content (from 6 to 550 and 60 mg N kg^{-1} respectively) and the 71% of aquifers sampled exceeded 45 mg l^{-1}. Our results indicate that uan application produced great N losses and did not increase soil residual N, suggesting that the high amount of nitrates in aquifers would arise from the soil N losses.

Keywords: Nitrate dynamics, microbial processes, water pollution.

INTRODUCTION

Agriculture expansion in Argentina has been highly significant in the last 15 years due to a strong global demand favored by the exchange rate as well as higher humidity in marginal areas, which promoted the expansion of agricultural frontiers. Such advantages provided higher economic incomes, which promoted the development of advanced crop technology. The most significant technological advance was the no-tillage (NT) use, associated with new agricultural machines, transgenic crops and agrochemical application [1].

Agrochemicals are heavily applied in Argentina at present because they are still relatively inexpensive and their use guarantees high yields. While there are regulations on the use of pesticides (herbicides, fungicides and insecticides) because of the direct risks to human health, the use of fertilizers has not been regulated yet (although they are not innocuous products). In Argentina, the commercialization of fertilizers is very important and increasingly expanding. Every year, new products and changes in technologies (doses and application timing) are introduced with the aim of increasing yields, but their effects on soil and water have been scarcely evaluated [2].

The effect of N fertilizers on soil in relation to amount and persistence of available N has been largely studied [3-5]. However, the modifications they produce on the dynamics of soil microorganisms are poorly understood. Soil microorganisms are important since their activity determines N availability for the crop and the possible losses by leaching or volatilization [6, 7].

Fertilizer N losses are a great global concern, since they alter atmospheric composition and water quality [8, 9]. The high nitrate content in water produces two severe environmental and sanitary problems: eutrophication and methemoglobinemia [10]. Eutrophication is a very common problem in water bodies near populated areas in Argentina and worldwide. This process consists in an excessive growth of algae and other aquatic plants due to an increase of nutrients in water. This increase in biomass is associated with a high rate of bacteria decomposition and consequent high reduction of dissolved O_2. Therefore, the main consequences associated with eutrophication are mortality of aquatic fauna, especially fishes, the high cost of drinking water treatment because of the great alga biomass, and the risks of toxicity when algae such as *Microcystis* spp. bloom [10, 11].

Methemoglobinemia is a disease caused by the consumption of water with high nitrate content: > 45 mg l^{-1}, according to the World Health Organization (WHO) and the U.S. Environmental Protection Agency (EPA). Nitrate replaces hemoglobin-O_2 and produces a reduction in tissue oxygenation. The condition is known as the “blue babies” syndrome because of their cyanotic skin. High doses of nitrates in water may cause death, especially in populations at high risk, like infants [12].

The Humid Pampa is an extensive agricultural area in the central-eastern of Argentina, which high levels of nitrates have already been reported in its aquifers [13]. However, the relationship between nitrate in aquifers and N losses driven by fertilizer applications has been scarcely evaluated [1, 2, 7]. The two factors that might contribute to potential N losses in agricultural soils in the Humid Pampa are: a) NT system, since it has been demonstrated that a great proportion of fertilizers is lost through leaching and volatilization when NT is utilized [1, 3, 14, 15]; and b) application of N fertilizers at sowing. The lack of synchronization between N availability and plant requirements in areas with abundant rainfall is the main cause of N leaching to aquifers [16].

Considering the advance of agriculture and fertilization in Argentina and the poorly evaluated associated environmental risks, the aim of this work was to determine the im-

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pact of N fertilizers on soil, especially on dynamics of microorganisms involved in nitrate release, and the possible relationship between the N losses and nitrate concentration in aquifers of the Humid Pampa of Argentina.

MATERIALS AND METHODS

Study Area

The study was conducted in two agricultural farms in Marcos Juárez (central agricultural area of Argentina) (32°45´S and 62°10´W), during the 2005-2006 maize crop cycle. The climate in the region is temperate sub-humid without water deficit, with a monsoon precipitation regime (annual precipitations between 850 and 1000 mm). Soils (Typic Argiudolls) are slightly acid, deep, rich in organic matter (4 %), and well drained. The water table ranges between 2-8 m in depth in the area.

Two different situations were evaluated (the most commonly fertilization schemes used in the region), in two sites (5 km between each other) with similar topographic and soil characteristics, and cropping system (NT in wheat-maize-soybean sequence). The fertilization situations analyzed were:

UAN treatment: 100 kg ha^{-1} of monoammonium phosphate (11 kg N and 22.88 kg P) applied in the seed line at sowing, and 230 l ha^{-1} of uan (urea, ammonium nitrate and ammonium tiosulfate: N 32% and S 5%) applied at five-leaf stage of growth (35 days after sowing). Total N: 110 kg ha^{-1}.

UREA treatment: 130 kg ha^{-1} of simple calcium superphosphate (P 20.37%, Ca 20%) and 70 kg ha^{-1} of calcium sulphate (S 18.6%, Ca 23.4%) at sowing and 120 kg ha^{-1} of urea (N 46%) applied at seven days after sowing. Total N: 60 kg ha^{-1}. In both situations maize was sowed in September and harvested by the end of March.

Sampling Design

Three composite samples (10 subsamples) of soil (0-30 cm in depth) were randomly taken in each site in an area of about 1 ha in the centre of each maize crop plot (approximately 50 ha). Sampling dates were: a) before sowing; b) one day after sowing; c) one day after application of N fertilizer, d) on two dates with a 15-days interval after fertilizer application; e) at flowering; and f) at harvest. In UREA treatment, sampling at sowing and at fertilizer application were concurrent. At harvest, 14 water samples were taken from the two most frequently used aquifers in the region: the water table (6-8 m) and the groundwater at 120 m, from wells present in an area of approximately 4000 ha that include the two study fields.

Chemical and Biological Analysis

Soil samples were air-dried and sieved through a 2-mm mesh. The following parameters were determined: pH (p/v 1:1); organic matter content (SOM) following Walkley and Black technique [17]; total N by micro Kjeldahl; nitrate by colorimetric method [18]; water content by gravimetric method; humic and fulvic acids following Adani *et al*. [19]; and abundance of microbial functional groups: ammonifier, cellulolytic, nitrifiers [20] and N-fixing microorganisms [21]. Nitrate content in water samples was determined through colorimetric method [18].

Data Analysis

Data of each treatment (UREA and UAN) were analyzed through ANOVA and Tuckey test to compare means between sampling dates ($P < 0.05$).

RESULTS

In UAN treatment, soil nitrate content showed the highest significant value at one day after uan application and the

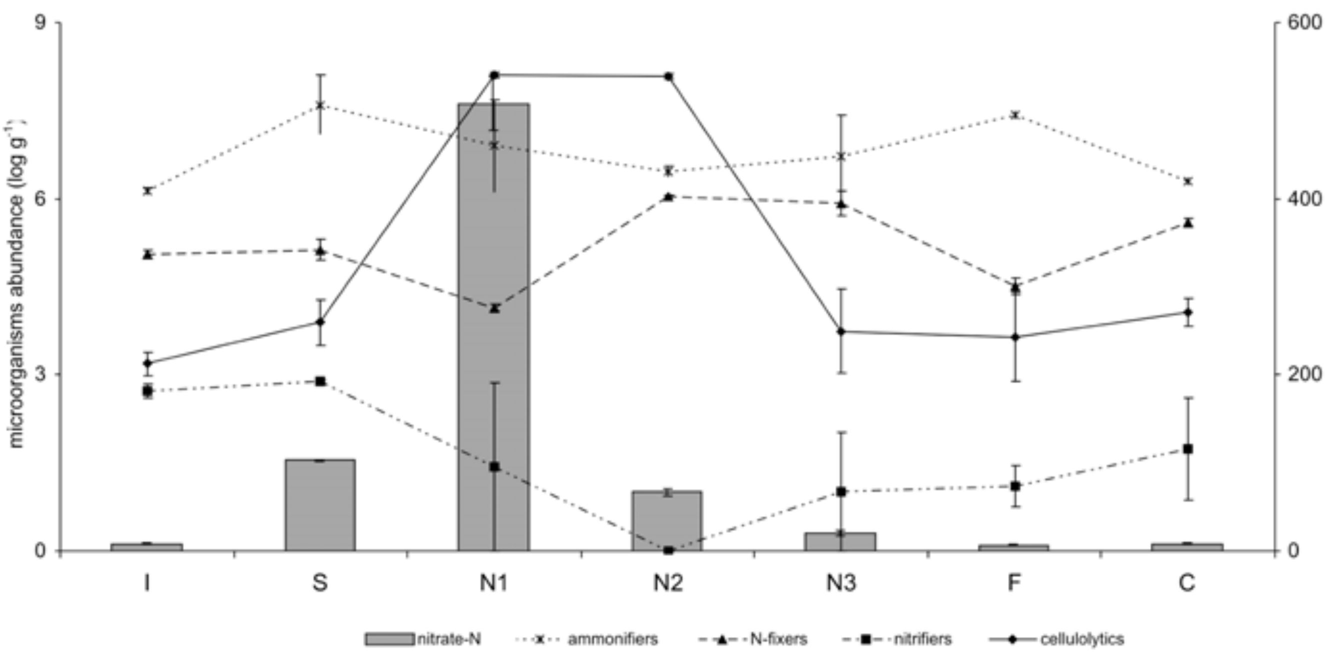

Fig. (1). Nitrate-N and microbial functional groups dynamics in maize plot fertilized with UAN treatment (110 kg N ha^{-1}). (I) before sowing; (S) one day after sowing; (N1) one day after uan application; (N2) 15 days after uan application; (N3) 30 days after uan application; (F) at flowering; and (C) at harvest. Bars corresponding to Standard Error.

Table 1. Soil Moisture, Fulvic Acids, Humic Acids, pH, Organic Matter, Total N, and C/N Ratio in the Maize Plot Fertilized with UAN Treatment (110 kg N ha^{-1}). (I) Before Sowing; (S) One Day After Sowing; (N1) One Day After Uan Application; (N2) 15 Days After Uan Application; (N3) 30 Days After Uan Application; (F) at Flowering; and (C) at Harvest. For Each Parameter, Different Letters Indicate Significant Differences Among Sample Dates (Tukey Test P>0.05)

	I	S	N1	N2	N3	F	C
Soil moisture (%)	17.30 bc	13.40 bc	19.19 ab	14.32 ab	23.14 ab	7.97 c	24.09 a
Fulvic acids (%)	0.29	0.27	0.34	0.26	0.36	0.27	0.34
Humic acids (%)	0.39	0.48	0.72	0.57	0.64	0.68	0.60
pH	5.85 c	5.90 c	6.01 bc	5.85 c	5.99 bc	6.14 ab	6.34 a
Organic matter (g kg^{-1})	41.9	46.7	42.8	33.6	45.0	28.7	35.7
Total N (g kg-1)	3.3	4.2	3.1	2.8	2.6	2.5	3.1
C/N	7.48	6.83	8.10	7.79	10.32	6.88	6.67

lowest values before sowing, at flowering and at harvest. Values of nitrates at sowing were also significantly higher than before sowing in agreement with the starter application of ammonium phosphate (Fig. **1**). Abundance of nitrifiers was highly influenced by UAN treatment, reaching non-detectable values at 15 days after uan application. In contrast, cellulolytic organisms increased significantly in the two samplings after fertilizer application. N2 fixing organisms showed a more erratic behaviour; although they decreased after fertilization, they showed another low peak value at flowering. In all cases, abundance of functional groups at the end of the crop cycle was similar to initial values before sowing (Fig. **1**), whereas at harvest, pH values were significantly higher than before sowing (Table **1**). Fulvic acids, humic acids, SOM, total N, and abundance of ammonifiers did not vary throughout the crop cycle.

In UREA treatment, soil nitrate content increased after sowing, reaching a maximum value at 30 days after fertilizer application and decreasing at flowering and harvest, with similar values to initial soil (Fig. **2**). Abundance of ammonifiers increased after sowing and remained stable until harvest, whereas N2-fixing organisms were lower at 15 days after urea application and higher at 30 days after urea application. The nitrifiers were significantly lower at 15 and 30 days after urea application and at flowering, and recovered their initial values at harvest (Fig. **2**).

Humic and fulvic acids were significantly lower at 15 days after fertilizer application, whereas no variation was

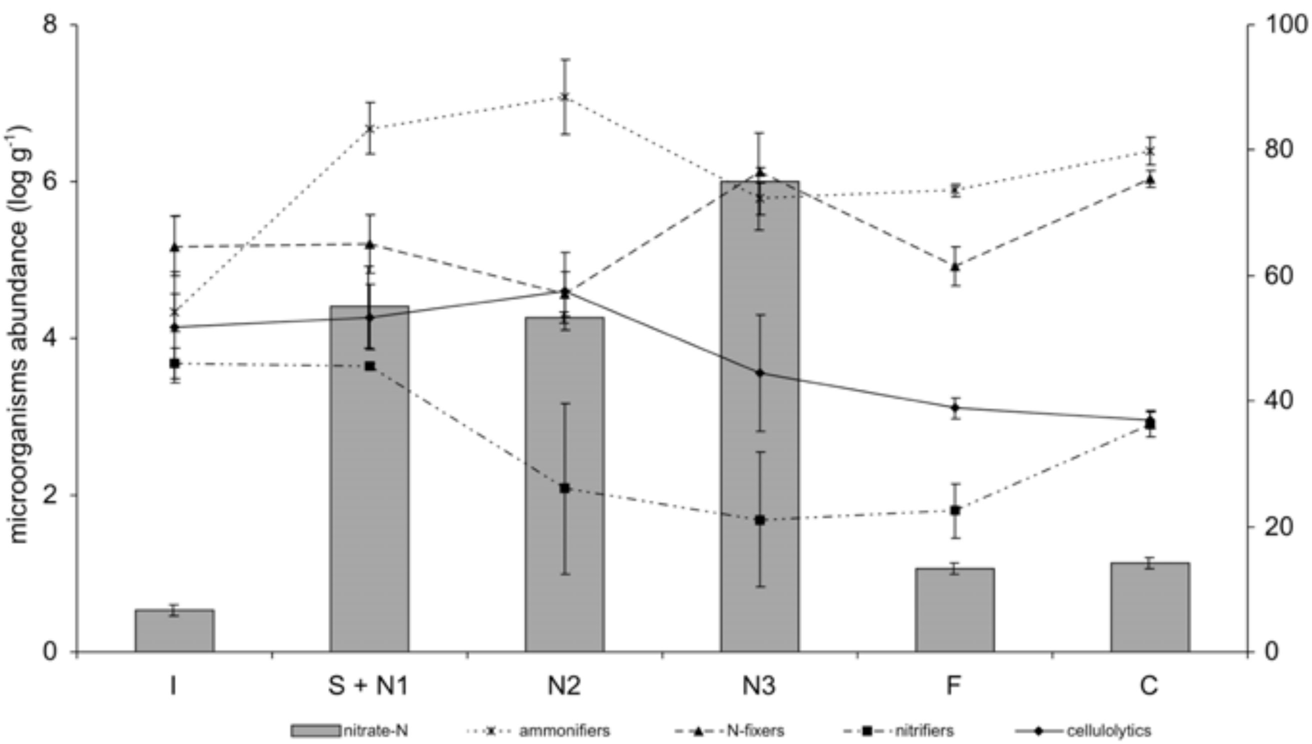

Fig. (2). Nitrate-N and microbial functional groups dynamics in maize plot fertilized with UREA treatment (60 kg N ha^{-1}). (I) before sowing; (S+ M1) one day after urea application at sowing; (N2) 15 days after urea application; (N3) 30 days after urea application; (F) at flowering; and (C) at harvest. Bars corresponding to Standard Error.

Table 2. Soil Moisture, Fulvic Acids, Humic Acids, pH, Organic Matter, Total N, and C/N Ratio in the Maize Plot Fertilized with UREA Treatment (60 kg N ha-1). (I) Before Sowing; (S+ M1) One Day After Urea Application at Sowing; (N2) 15 Days After Urea Application; (N3) 30 Days After Urea Application; (F) at Flowering; and (C) at Harvest. For Each Parameter, Different Letters Indicate Significant Differences Among Sample Dates (Tukey Test P>0.05)

	I	S+ N1	N2	N3	F	C
Soil moisture (%)	18.60 b	18.44 b	16.39 b	25.01 a	15.99 b	23.11 a
Fulvic acids (%)	0.34 ab	0.43 ab	0.25 c	0.31 b	0.36 ab	0.49 a
Humic acids (%)	0.69 a	0.64 a	0.14 c	0.47 b	0.73 a	0.59 a b
pH	6.15	6.40	6.50	6.27	6.25	6.12
Org. matter (g kg^{-1})	39.1	39.2	21.6	30.1	31.8	27.2
Total N (g kg-1)	39.1	39.2	21.6	30.1	31.8	27.2
C/N	11.80	6.33	5.45	6.84	7.56	5.57

observed in pH, SOM, total N, or abundance of cellulolytics (Table **2**).

Nitrate Content in Aquifers

Of the 14 wells analyzed, 71.5% exhibited nitrate values higher than those allowed by WHO and EPA (45 mg L-1). Seven of the eight water samples from the water table (8 m) were above the maximum recommended level, whereas half of the water samples from 120 m in depth exceeded this value (Table **3**).

Table 3. Aquifer Nitrate Content in Marcos Juarez (Cordoba Province). Numbers (from #1 to #14) Corresponding to Samples Number. In Bold Values Higher than EPA and OMS Recommended Level (45 mg l-1)

Groundwater (120 m in Depth)		**Water Table (6- 8 m in Depth)**	
Nitrate (mg L^{-1})			
#1	1.3	# 7	5.0
#2	0.0	# 8	**14.0**
# 3	**61.7**	# 9	**171.0**
# 4	**54.5**	#10	**189.0**
# 5	**56.5**	# 11	**81.0**
# 6	22.7	#12	**103.0**
		# 13	**105.5**
		#14	**151.0**

DISCUSSION

Effect on Soil Dynamics

Our results indicate that the effect of nitrogen fertilization on soil N dynamics in maize crops varies greatly with: a) fertilizer chemical composition, b) application timing, and c) N doses. In general, these observations agree with other authors' statements, although few works report the short-term effect of fertilizers on soil microbial populations [4, 16, 22].

In our study, the chemical composition of the fertilizer clearly defines the amount and persistence of nitrate released. In the fertilizer with organic N form (urea), the increase in nitrate concentration 24 h after application (10 times higher than the initial value) persists for approximately 30 days and decreases abruptly after 30-76 days, at the start of plants' flowering stage. This nitrate dynamics is related to important changes in microbial activity due to: a) the presence of organic N (increase in ammonifiers) and b) the feedback effect of soil nitrate (decrease of nitrifiers) [23, 24].

In UAN treatment, the increase of nitrates 24 h after application of ammonium-based fertilizer (ammonium phosphate at sowing) is similar to that in UREA treatment (14 times greater than initial soil), but is not related to changes in microbial populations involved in the N cycle, probably because of the low amount of ammonium applied (11 kg N ha^{-1}).

Uan fertilizer, based on three chemical N forms (nitrate, ammonium and urea), has a very important impact on soil. The increase of nitrates 24 h after application (61 times greater than initial soil) has a strong negative effect on abundance of N_2-fixing organisms and nitrifiers (which decrease to non-detectable values 15 days after application) and a positive effect on cellulolytic organisms.

The increase in cellulolytic organisms would be indicating that high N availability favours the growth of microbial populations related to crop residues degradation [25]. This fact disagrees with the general idea that high rates of fertilizers contribute to the increase of SOM because they produce higher amount of crop residues. When N fertilizer exceeds plant requirements, C from crop residues may be lost by the increase of biomass and microbial activity [7, 26, 27]. This statement is consistent with the lack of increase in SOM content and humic substances at the end of the crop cycle found in this study.

Consequently, the two greatest differences detected in soil N dynamics between UREA and UAN treatments are: a) higher amount of nitrates released in UAN treatment, and b) greater persistence of nitrates in UREA treatment. The fact that the highest nitrate values from UAN decreases abruptly in a short period (from 508 mg N kg^{-1} to 66 mg N kg^{-1} in 15 days) has important practical implications in relation to the crop requirements, N residual management and nitrate losses to the groundwater [4, 16].

Synchronization with Crop Requirements

One of the most controversial aspects in fertilizer application is synchronization with crop requirements [16, 28]. According to Andrade *et al.* [29], Humid Pampa maize crop requires 3.7 kg N d^{-1} from the 25^{th} day after emergence. Based on this information, we can estimate the correspondence between nitrate demand and availability for each fertilizer evaluated. In UREA treatment, during the period between 15 and 30 days after urea application the crop required 66 kg N ha^{-1} and had an availability of 106 kg N ha^{-1}, whereas between 30 days after urea application and flowering, the crop needed 130 kg N ha^{-1} and had 140 kg available N ha^{-1}. In contrast, in UAN treatment, between one and 15 days after uan application the crop required 26 kg N ha^{-1} and had 1016 kg available N ha^{-1}; between 15 and 30 days after uan application the crop required 52 kg N ha^{-1} and had 133 kg available N ha^{-1}, and between 30 days after uan application and flowering, it required 207 kg N ha^{-1} and only had 40 kg available N ha^{-1}.

In Argentina N fertilizer application is usually recommended at five-leaf stage of plant growth with the aim of improving synchronization with crop requirements [30]. The huge availability of nitrates in UAN treatment is observed when the plant barely needs it, and the great subsequent decrease might indicate N deficit prior to flowering. This observation would be consistent with: a) a lower yield obtained in the plot treated with uan (10500 kg ha^{-1}) than fertilized with urea (11700 kg ha^{-1}), and b) the increase of N_2-fixing organisms when nitrate content decreases and crop requirement increases. It is well known that plants and microorganisms compete when soil available N is scarce, which favours microorganisms that can use atmospheric N_2 [27].

Residual N

Our results show that none of the fertilizers used added residual N to the soil: values of total N and nitrates at the end of the crop cycle were similar to those from pre-sowing. The fact that no differences were detected between fertilizers is surprising, since the UAN treatment received almost double doses of N than the UREA treatment (110 kg N ha^{-1} *vs* 60 kg N ha^{-1}).

The lack of residual N cannot be due to a greater uptake by the crop in UAN treatment because crop yield was lower than in UREA treatment. This observation would be related to the usually high precipitations in the area during the crop cycle. Most reports of higher N residuality are from arid zones or irrigated areas, or where irrigation is interrupted at the end of the crop growth cycle [16, 31].

N Losses to the Aquifers

The climatic conditions in Marcos Juárez favour N losses through leaching to the groundwater: abundant rains during the crop cycle and very shallow water table (6-8 m). During the crop cycle evaluated rainfall amounted to 700 mm, concentrated in November (150 mm), when nitrate availability is high and plant requirements are low. This observation is consistent with the high nitrate content detected in the groundwater during this study.

The amount of nitrate N potentially leached from the fertilizer plots to the groundwater can be estimated using: a) data obtained in November, because of the heavy precipitations and low plant N requirements; and b) the amount of nitrates not used by the plant (difference between available and required nitrates). The calculation results in 40 kg N ha^{-1} potentially leached in the plot treated with UREA and 1000 kg N ha-1 with UAN. This very high value agrees with values estimated in a work on N balance in wheat, when 140 kg N ha^{-1} was applied [7] and with the high amount of fertilizer $_{15}N$ detected by Power and Peterson [14] in deep soil horizons.

Because of the magnitude of this problem in Argentina, more rational fertilization management strategies should be implemented without compromising crop yields. This is a concern in several producing countries where the use of fertilizers is regulated in different ways, depending on the area, crop type, climatic characteristics, and use of groundwater [5, 28].

In summary, our results on the effect of N fertilization on soil N dynamics and its relationship with nitrate contents in aquifers indicate that: a) uan fertilizer has a important effect on soil, producing great losses and less benefits to the plant; b) high doses of fertilizers do not increase soil residual N; and c) a high amount of nitrates is lost from the surface horizon, which would be related to the high nitrate content detected in aquifers. Therefore, a rational use of nitrogen fertilizers may benefit soil sustainability, water quality and producers' profitability.

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