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Variation in Dissolved Oxygen Levels Between Agricultural and Protected Streams in Loudoun County, Virginia

Shray Vij[1,*] and Ryan S. Nisay[2]

[1, 2]Research Division, Loudoun Nature Conservation Project, 20166, VA, USA, [1, 2]Potomac Falls High School, 20165, VA, USA, [1, 2]Academies of Loudoun, 20175, VA, USA and [2]Cornell University, 14850, VA, USA

*Corresponding author. vijshray09@gmail.com

Abstract

Agricultural runoff can reduce dissolved oxygen (DO) in streams and increase the risk of hypoxia for aquatic organisms. We monitored four streams in Loudoun County, Virginia for four weeks to compare DO dynamics between two agricultural sites and two protected forested sites, and to observe the response to a 1.3 inch storm event. Sites were sampled twice daily for DO and related water quality parameters, with weekly nutrient samples. Agricultural streams had substantially lower mean DO than protected streams and a larger share of measurements below the 5.0 mg/L stress threshold. After the storm, DO in agricultural streams dropped sharply while protected sites remained within healthy ranges, suggesting reduced resilience to runoff pulses. These results indicate that agricultural land use is associated with chronic DO impairment and greater vulnerability to storm-driven hypoxia, and they support management strategies such as riparian buffer restoration and nutrient and runoff control.

Key words: dissolved oxygen, hypoxia, agricultural runoff, eutrophication, nitrite toxicity, nitrate pollution, freshwater ecosystems, oxidative stress

Introduction

Dissolved oxygen (DO) is an important indicator of water quality and the capacity of a freshwater body to support aquatic life (1). While DO levels fluctuate naturally, anthropogenic pressures or agricultural pollution can cause depletion, known as hypoxia (2). When DO concentrations drop below the critical threshold of 5.0 mg/L, most aquatic organisms experience physiological stress, reduced reproduction, and mortality, leading to communities dominated by a few pollution-tolerant species (1; 5).

An important mechanism for depletion is cultural eutrophication. Nutrient-rich runoff, particularly nitrogen and phosphorus from fertilizers and animal waste, fuels massive algal blooms (3).. The subsequent decomposition of this organic matter by aerobic bacteria consumes large quantities of dissolved oxygen, creating hypoxic and anoxic zones (4). This process is a documented global issue (6) and a driver of ecological impairment in watersheds, including the Chesapeake Bay, where agricultural runoff accounts for a significant portion of the total nutrient load (8). Specifically, agriculture contributes 41% of the nitrogen load and 48% of the phosphorus load to the Chesapeake Bay.

Loudoun County, Virginia, represents a critical example of this issue, with expanding urbanization encroaching on established agricultural lands. Local monitoring data indicate that streams in agricultural watersheds consistently exhibit lower DO concentrations and higher nutrient loads than protected, forested streams (9). While chronic stress is well known, the vulnerability of these streams to acute, storm-driven pollution events remains unquantified. It is unclear how resilient these agricultural streams are compared to their protected counterparts.

This study aims to fill that gap by providing a direct, medium-frequency comparison of DO dynamics in four Loudoun County streams. This involves comparing two sites impacted by agricultural land use with two protected reference sites. By capturing both baseline conditions and the impact of a 1.3-inch storm event, this research quantifies the true extent of dissolved oxygen's impacts and the loss of ecological resilience caused by agricultural runoff.

Previous Studies on Dissolved Oxygen

Background

Dissolved oxygen (DO) serves as one of the most critical indicators of freshwater ecosystem health. It influences nearly every aspect of aquatic ecology, from respiration and metabolism to biodiversity and nutrient cycling. Variations in DO levels can indicate the balance between oxygen production through photosynthesis and its consumption during decomposition and respiration. Human-induced changes to watersheds, such as agricultural runoff and deforestation, disrupt this equilibrium by increasing nutrient and sediment loads in streams (1) (2).

In natural freshwater systems, oxygen concentrations fluctuate due to factors such as water temperature, flow velocity, and organic content. However, anthropogenic disturbances intensify these fluctuations, often leading to hypoxic conditions. Several studies have linked nutrient enrichment and eutrophication to widespread oxygen depletion and ecological stress (3) (4). These findings form the foundation for understanding the causes of low DO levels in both agricultural and protected streams.

Dissolved Oxygen and Freshwater Ecology

Dissolved oxygen levels are a key determinant of ecosystem productivity and the survival of aquatic organisms (3). Oxygen scarcity impairs physiological functions in fish and invertebrates, leading to metabolic depression and altered community composition. Spatial and temporal variation in DO reflects the broader health of freshwater systems, where even minor fluctuations can signal changes in water chemistry, habitat structure, or nutrient load (4). Together, DO is established as a reliable biological and chemical indicator of environmental integrity.

Impact of Nutrient Pollution and Runoff on DO

Agricultural runoff contributes significantly to nutrient enrichment in aquatic systems. Excessive inputs of nitrogen and phosphorus from fertilizers accelerate eutrophication, leading to harmful algal blooms and severe oxygen depletion (5). As algae proliferate and decompose, microbial respiration consumes dissolved oxygen at rates faster than replenishment. This sequence produces hypoxic conditions that stress aquatic life and degrade water quality. Studies also indicate that nitrate and nitrite accumulation disrupts oxygen balance by fueling heterotrophic activity and altering redox conditions near streambeds (2)(5).

Stream Health Indicators and Monitoring Methods

Modern monitoring frameworks use a combination of physicochemical and biological indicators to evaluate stream health. Riparian buffers stabilize DO by filtering nutrient runoff, reducing sedimentation, and shading streams to moderate thermal stress (6). Studies demonstrate that best management practices (BMPs)—including vegetated buffers, cover crops, and retention basins—enhance water quality and increase stability of DO (7). Continuous monitoring of DO, pH, turbidity, and macroinvertebrates populations offers an effective approach to tracking restoration results and detecting nutrient-related degradation in real time.

Restoration Strategies and Chesapeake Bay Recovery

The Chesapeake Bay Program and the U.S. Environmental Protection Agency (EPA) have implemented extensive restoration programs to address low levels of DO across the watershed. Long-term data show that nutrient reduction policies and agricultural BMPs have improved oxygen conditions in multiple tributaries (8). These strategies include riparian restoration, wetland reconstruction, and controlled fertilizer application. The Chesapeake Bay Foundation reports emphasize that sustained reductions in nitrogen and phosphorus loads correlate directly with recovery of dissolved oxygen and overall aquatic resilience (8).

Materials and Method

Dissolved oxygen (DO) concentrations are quantified and compared in agricultural streams and protected forested reference streams in Loudoun County, Virginia, through this methodology. The methodology was designed to isolate the depression of DO driven by nutrients, following standard freshwater water quality protocols.

Four stream locations were selected. Two were classified as protected reference sites with intact riparian forest buffers and minimal anthropogenic disturbance. Two were agricultural sites directly influenced by crop production and livestock access. The sites were selected based on the characteristics of the watershed, nutrient potential, and the integrity of the buffer width.

Protected Sites:

- Defined as having more than 300 ft of continuous forested riparian buffer and negligible upstream agricultural use.
  - P1: Catoctin Creek [39.411694 °N, -77.571503 °W]
  - P2: Goose Creek [32.981000 °N, -80.032583 °W]

Agricultural Sites:

- Defined as having discontinuous riparian buffers (less than 50 ft) and direct runoff paths from active crop fields or livestock pasture.
  - A1: Little River [33.877258 °N, -78.617477 °W]
  - A2: Foley Branch [38.909608 °N, -77.553384 °W]

Fig. 1: Map of the Catoctin Creek sampling location (P1) in Loudoun County, Virginia.

Catoctin Creek Data Collection

Figure 1 shows the point of data collection near a public water access on the stream. The specific point for data collection was chosen to be accessible, mid-stream, and away from any trail crossings to minimize localized disturbance from park users. Furthermore, this location serves as an example for a protected site by being more than 300 feet away from riparian buffer.

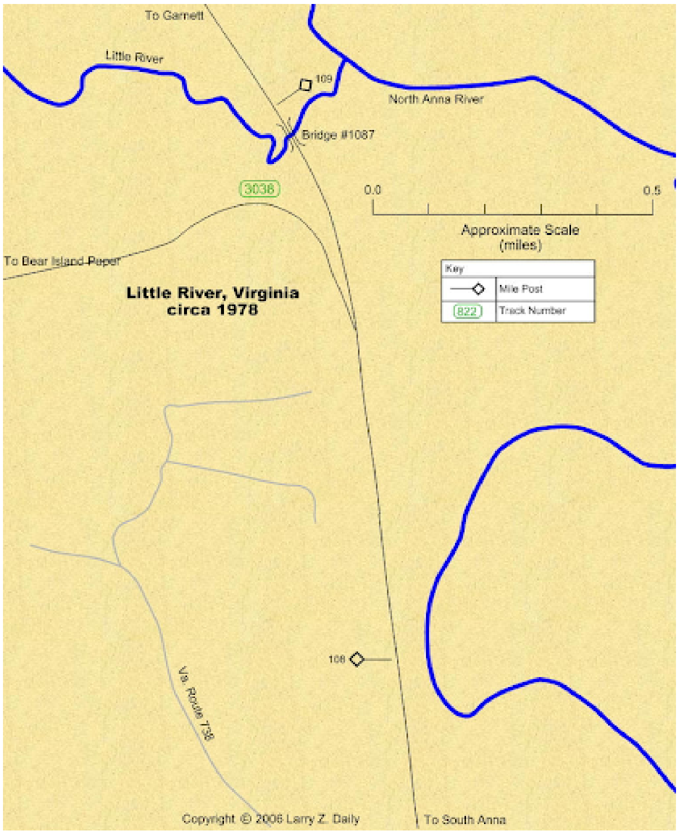

Fig. 2: Map of the Little River sampling location (A1) showing surrounding agricultural land use.

Little River Data Collection

Figure 2 shows the sampling location near the intersection of the river and a main road, immediately upstream or downstream of the Bridge #1087 near the VA Route 3038 intersection. This site was selected because the upstream land use is characterized by active crop production and minimal riparian buffer width (less than 50 ft), making it highly susceptible to non-point source pollution, particularly during storm events. The location was chosen to directly capture runoff impacts before significant mixing or dilution occurs.

Assessments and Measures

Sampling was conducted during a 28-day period from October 1 to October 28, 2025. This timeframe was chosen to check both baseline differences and response to a major rainfall event. Each of the four sites was sampled twice daily. This involved a measurement in the morning 08:30 - 10:30 AM EST and afternoon 14:00 - 16:00 PM EST. This method of measurement effectively captures typical fluctuations during the day. This protocol resulted in 224 primary sampling events, with an additional sampling round conducted 24 hours after a 1.3 inch precipitation event, yielding a total of 228 sampling events for the study. At each site during every event, in-stream measurements were taken in triplicate (spatial replication) at the point of maximum flow. These triplicates were averaged to produce a single data point for the event.

All measurements were taken mid-stream at a depth of approximately 15 cm. DO concentration (mg/L), percent saturation (%), and water temperature (°C) were recorded using a YSI Pro20i handheld meter. The meter was calibrated daily using the manufacturer's air-saturated water protocol and maintained an accuracy of 0.1 mg/L for dissolved oxygen. Conductivity (uS/cm) was measured with a Hanna HI98129 multiparameter pocket meter, calibrated daily to a 1413 uS/cm standard solution. Turbidity (NTU) was measured from grab samples using a LaMotte 2020we turbidity meter. For nutrient analysis, 100 mL grab samples were collected, immediately placed on ice, and transported to the lab. Samples were filtered through a 0.45-um filter and chemically preserved by acidifying with 1 mL of concentrated Sulfuric Acid to a pH ¡ 2 before storage at 4 °C. All nutrient samples were analyzed within the 24-hour holding time using Hach DR/3900 colorimetric kits for nitrate and phosphate.

Data Analysis

All data were organized in Microsoft Excel and processed in **R (Version 4.4.0)**. Descriptive statistics, including mean, minimum, maximum, and standard deviation, were calculated for all parameters across the pooled "Protected" (P1, P2) and "Agricultural" (A1, A2) datasets. Initial comparisons of means between agricultural and protected sites were evaluated using a two-sample t-test treating repeated observations as independent, which we used as an exploratory comparison despite the repeated-measures structure of the data. Further analysis utilized a Two-Way Analysis of Variance (ANOVA) to explore the interaction between Site Type (Agricultural/Protected) and Time of Day (Morning/Afternoon) on dissolved oxygen concentration, and a paired t-test was employed to quantify the DO response to the storm event. To quantify the strength and direction of the linear association between the variables, Pearson Correlation Coefficients (r) were calculated for DO concentration (mg/L) against Nitrate, Turbidity, and Temperature, with coefficients calculated separately for the Agricultural and Protected sites. Pearson correlation coefficients, which yield values between -1.0 and +1.0 indicating the strength of the linear relationship, were calculated. For all tests, a standard alpha level of $a = 0.05$ was used, representing the conventional 95 percent confidence level typically utilized in ecological field studies.

Because the same sites were sampled repeatedly, these inferential tests should be interpreted with caution and viewed as exploratory rather than fully meeting the assumption of independent observations.

Results

We conducted a high-frequency comparative stream study across four sampling sites, grouped into two land-use categories: protected (P1, P2) and agricultural (A1, A2). This focused sampling yielded 228 total measurements over the 4-week period. The analysis of this data, presented in the figures below, reveals an impairment of water quality associated with agricultural land use.

Site (ID)	Site (Type)	Date	Nitrate (mg/L)	Phosphate (mg/L)	DO (mg/L)	Turbidity (NTU)	Temperature (°C)
P1	Protected	2025-10-16	0.9	0.07	8.5	10.5	17.5
P2	Protected	2025-10-16	0.8	0.06	8.6	11.2	17.4
A1	Agricultural	2025-10-16	4.5	0.41	5.4	55.1	18.2
A2	Agricultural	2025-10-16	5.1	0.48	5.1	61.4	18.3
P1	Protected	2025-10-23	1	0.08	8.2	9.9	16.9
P2	Protected	2025-10-23	0.9	0.07	8.3	10.3	16.8
A1	Agricultural	2025-10-23	4.8	0.45	5.1	51	17.5
A2	Agricultural	2025-10-23	5.3	0.5	4.8	58.2	17.6
P1	Protected	2025-10-26	1.1	0.09	7.9	12.1	16.2
P2	Protected	2025-10-26	1	0.08	8	11.8	16.1
A1	Agricultural	2025-10-26	5	0.47	4.9	59.5	17
A2	Agricultural	2025-10-26	5.5	0.52	4.6	65	17.1
P1	Protected	2025-10-30	0.8	0.06	8.8	9	18
P2	Protected	2025-10-30	0.7	0.05	8.9	8.8	17.9
A1	Agricultural	2025-10-30	4.3	0.39	5.8	49	19.1
A2	Agricultural	2025-10-30	4.9	0.46	5.5	53.4	19.2
P1	Protected	2025-11-01	1.4	0.15	7.5	20.1	15.1
P2	Protected	2025-11-01	1.3	0.14	7.6	19.8	15
A1	Agricultural	2025-11-01	7.1	0.75	3.5	151.3	15.5
A2	Agricultural	2025-11-01	6.9	0.72	3.6	148.7	15.6

Fig. 3: Mean turbidity (NTU), nitrate (mg/L), and phosphate (mg/L) at protected (P1, P2) and agricultural (A1, A2) stream sites. Error bars show standard deviation.

Sample (ID)	Site (ID)	Site (Type)	Date	Time	Rain (Event)	DO (mg/L)	DO (%)	Temp (°C)	pH	Conductivity (uS)	Turbidity (NTU)
1	P1	Protected	2025-10-16	Morning	No	8.5	92.1	17.5	7.2	310	10.5
2	P1	Protected	2025-10-16	Afternoon	No	9.1	98.2	18.8	7.4	312	9.8
3	P2	Protected	2025-10-16	Morning	No	8.6	92.5	17.4	7.3	330	11.2
4	P2	Protected	2025-10-16	Afternoon	No	9.2	98.6	18.7	7.4	330	11
5	A1	Agricultural	2025-10-16	Morning	No	5.4	51.5	18.2	6.8	720	55.1
6	A1	Agricultural	2025-10-16	Afternoon	No	6.1	59	19.5	7	725	52
7	A2	Agricultural	2025-10-16	Morning	No	5.1	49	18.3	6.7	780	61.4
8	A2	Agricultural	2025-10-16	Afternoon	No	5.8	56.1	19.6	6.9	782	59.5
9	P1	Protected	2025-10-17	Morning	No	8.4	91.8	17.2	7.2	308	10.1
10	P1	Protected	2025-10-17	Afternoon	No	9	97.5	18.5	7.3	310	9.5
11	P2	Protected	2025-10-17	Morning	No	8.5	92	17.1	7.3	328	11
12	P2	Protected	2025-10-17	Afternoon	No	9.1	98.1	18.4	7.4	329	10.8
13	A1	Agricultural	2025-10-17	Morning	No	5.2	50.1	18	6.7	725	58.2
14	A1	Agricultural	2025-10-17	Afternoon	No	5.9	57.5	19.2	6.9	730	54.3
15	A2	Agricultural	2025-10-17	Morning	No	4.9	47.5	18.1	6.7	785	63
16	A2	Agricultural	2025-10-17	Afternoon	No	5.6	54.8	19.3	6.8	790	61.1
17	P1	Protected	2025-10-18	Morning	No	8.2	90.1	16.9	7.1	315	9.9
18	P1	Protected	2025-10-18	Afternoon	No	8.8	96	18.1	7.3	314	9.2
19	P2	Protected	2025-10-18	Morning	No	8.3	90.5	16.8	7.2	335	10.3
20	P2	Protected	2025-10-18	Afternoon	No	8.9	96.6	18	7.3	336	10.1

Fig. 4: Daily dissolved oxygen (mg/L), pH, temperature (°C), conductivity (µS/cm), and turbidity (NTU) at protected and agricultural stream sites over the 4-week study period.

The two-sample t-test on baseline data showed that the agricultural sites suffer from chronic hypoxia, recording a mean Dissolved Oxygen concentration of 5.40 +/- 0.95 mg/L (mean +/- SD}), which is a 38.1 percent reduction compared to the protected streams (8.71 +/- 0.45 mg/L), p ¡ 0.001). This oxygen depletion is paralleled by elevated pollutant indicators: baseline Conductivity was 137.3 percent higher (757 +/- 20 uS), and Turbidity was nearly five times higher (56.4 +/- 6.9 NTU) in the agricultural streams (p less than 0.001 for all). This strong difference in mean parameters suggests that land use might be an important factor driving the observed physicochemical conditions.

To investigate the statistical associations between DO and key environmental variables, Pearson Correlation Coefficients r were calculated across the entire dataset (n = 224) for Turbidity and Temperature, n = 16 for nutrients. This analysis is essential for identifying which factors are most closely correlated with DO loss. The results show that while stream temperature has a moderate negative correlation with DO (r = -0.48, p less than 0.001), the influence of agricultural indicators is substantially stronger. DO showed a negative correlation with Nitrate (r = -0.71 [n = 16] , p less than 0.001) and a moderate-strong negative correlation with Turbidity (r = -0.65 [n = 224], p less than 0.001). These associations provide evidence that DO depletion has some linkage to the introduction of high nutrient loads and suspended solids, supporting a mechanism consistent with cultural eutrophication. It is important to note that correlation demonstrates association, not definitive causation.

The vulnerability of the agricultural streams was exposed during the post-storm sampling event (n = 8). This stress test demonstrated afailure in watershed resilience, with mean DO in agricultural sites plummeting from 5.11 +/- 0.2 mg/L to a low 3.40 +/- 0.2 mg/L (Paired t-test, p = 0.006). This crash coincided with a massive influx of pollutants, evidenced by Turbidity spiking to 151.77 +/- 3.0 NTU (a 2.7x increase from the already high baseline). In contrast, the protected sites maintained water quality within the healthy range. This evidence supports the hypothesis that agricultural land use results in streams that are both stressed and susceptible to acute hypoxic events triggered by rainfall-driven runoff.

The analysis utilized data from two primary sources to derive specific statistical results. The large high-frequency dataset (n = 224), represented by Figure 4, included measurements for DO, conductivity, turbidity, and pH and was used to establish baseline differences between protected and agricultural sites. This same dataset was used to calculate the Pearson correlation coefficients linking DO to both turbidity (r = -0.65) and temperature (r = -0.48). Separately, the nutrient dataset (n = 16), represented by Figure 3, was used to quantify the increase in nutrient concentrations and to calculate the correlation between DO and nitrate (r = -0.71). Finally, a specific subset of eight measurements was extracted from the high-frequency dataset (Figure 4) to perform the paired t-test and calculate the means for the post-rainfall resilience analysis.

Discussion

While our study provides strong statistical evidence of chronic hypoxia and reduced resilience in streams associated with agricultural land use, several limitations related to data collection and experimental design must be acknowledged.

Our results suggest that agricultural land use is associated with both chronic DO impairment and reduced resilience to storm events. Agricultural streams had lower mean DO and a greater share of measurements below the 5.0 mg/L stress threshold than protected streams, indicating that baseline conditions in these waters are already close to physiological limits for sensitive species. The sharp DO decline in agricultural streams after the 1.3 inch storm, contrasted with stable DO in protected streams, shows how storm-driven runoff can push these already stressed systems into acute hypoxic events.

Mechanistically, this pattern is consistent with nutrient- and sediment-driven oxygen demand. Runoff from fields and pastures delivers nitrogen, phosphorus, and fine sediments to the stream. These inputs can fuel algal growth and microbial decomposition, which consume oxygen, while elevated turbidity and sediment reduce light penetration and photosynthesis. During storm events, pulses of organic matter and nutrients intensify these processes, causing rapid DO crashes before the system can recover. Agricultural sites with narrow or discontinuous riparian buffers are especially vulnerable because there is little vegetation to intercept or slow runoff before it reaches the channel.

These findings point to clear management implications. Wider and more continuous riparian buffers, livestock exclusion from streams, and practices that reduce stormflow and nutrient export (such as cover crops, retention basins, and more careful fertilizer application) are likely to improve DO conditions and increase resilience to storm events. Protecting and restoring forested buffers in headwater and mid-order streams may be particularly important in landscapes similar to the study area.

Temporal and Spatial Scope

The data collection period was limited to four weeks in October 2025, capturing only 14 field days with two daily measurements per site. This temporal scope may not fully represent seasonal fluctuations in Dissolved Oxygen (DO) dynamics, which are typically driven by summer warming or winter flow regimes. Additionally, the study was constrained to a limited spatial scale, relying on only two protected sites (P1, P2) and two agricultural sites (A1, A2). Compiling these four streams for statistical analysis reduces insight into site-specific variability and local geological or hydrological differences, constraining the generalizability of our findings beyond the specific streams studied. The lack of replication at the watershed scale prevents extrapolation of these results to other regions.

Instrument and Measurement Error

Instrumentation and measurement procedures introduced potential sources of error. DO readings were collected *in situ* using handheld meters, meaning minor inconsistencies in calibration, probe placement, or environmental conditions could have influenced individual measurements. Furthermore, nutrient analyses (Nitrate and Phosphate) were performed from weekly grab samples. This sampling frequency may have missed short-term, acute fluctuations in pollutant concentrations, particularly the peak sediment and nutrient pulses that occur immediately following storm-driven runoff events, potentially underestimating the true maximum concentrations. Similarly, turbidity measurements may not fully capture peak sediment pulses.

Statistical and Causal Inference

The use of Pearson correlation coefficients (r) and t-tests provided robust initial insight into the associations between agricultural runoff indicators and DO levels. However, these parametric methods assume independent measurements. Repeated measures from the same four sites over time may violate this assumption of non-independence. Most critically, while the strong correlation coefficients (e.g., r = -0.71 [n = 16]) demonstrate a close statistical relationship, they do not prove causation. Proving that nutrient inputs directly *caused* the hypoxia would require a controlled experimental manipulation or more complex time-series analysis that were beyond the scope of this study.

Contextual and Biological Omission

Environmental and contextual factors were not continuously monitored. Variations in stream flow, ambient temperature, and exact precipitation timing could have affected DO dynamics independently of agricultural inputs. The absence of comprehensive weather variability data limits our ability to fully contextualize storm-driven events. Finally, the absence of concurrent biological surveys, such as macroinvertebrate or fish community sampling, limits our ability to link the observed chemical changes directly to definitive ecological impacts. Therefore, the ecological severity of the agricultural impairment is inferred chemically (DO¡ 5.0 mg/L being the established proxy for aquatic stress).

This study rigorously compared the physicochemical water quality of streams associated with agricultural land use against protected reference streams. The analysis of the High-Frequency Dataset (n = 224) provides strong statistical evidence that land use differences are associated with hypoxia during the sampling period. Agricultural sites exhibited a mean DO concentration of 5.40 +/- 0.95 mg/L (mean +/- SD), representing a 38.1 percent reduction compared to protected streams (8.71 +/- 0.45 mg/L). This difference was confirmed as statistically significant (p less than 0.001) and was further supported by corresponding elevations in Conductivity and Turbidity at the agricultural sites.

Correlational analysis supports an association between DO depletion and agricultural inputs. DO showed strong negative correlations with Nitrate (r = -0.71 [n = 16], p less than 0.001) and Turbidity (r = -0.65 [n = 224] , p less than 0.001), consistent with the hypothesis of eutrophication-driven oxygen loss. Furthermore, the post-storm sampling revealed a diminished resilience in agricultural streams, which experienced a DO decline (p = 0.006) to low levels, suggesting vulnerability to runoff events. These findings establish that agricultural activity is associated with impairment and water quality declines.

The current study focused primarily on physicochemical measurements with single-point triplicates per event. However, this design did not include the level of replication that is beneficial for ecological interpretation. Future work is necessary to integrate both biological replication to provide ecological validation of chemical stress, and nested spatial replication (sampling multiple distinct locations within the same reach) to better account for heterogeneity and reduce sampling location bias.

Overall, these findings support further investigation into the precise mechanisms and temporal consistency of DO impairment in these watersheds. Future research must address the study's limitations by incorporating multi-season monitoring to capture temporal variability, including additional independent watersheds to increase spatial replication, and integrating biological community assessments to strengthen the ecological interpretation beyond chemical proxies.

Competing interests

We have no known conflict of interest to disclose.

Author contributions statement

S.V. and R.N. conceived the experiment(s), V.S. lead the data collection(s), V.S. analysed the results. V.S. wrote draft, R.N. and V.S. reviewed, edited, formatted manuscript.

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