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Nutrient limitation of phytoplankton in three tributaries of Chesapeake Bay: Detecting responses following nutrient reductions

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ABSTRACT

Many coastal ecosystems suffer from eutrophication, algal blooms, and dead zones due to excessive anthropogenic inputs of nitrogen (N) and phosphorus (P). This has led to regional restoration efforts that focus on managing watershed loads of N and P. In Chesapeake Bay, the largest estuary in the United States, dual nutrient reductions of N and P have been pursued since the 1980s. However, it remains unclear whether nutrient limitation – an indicator of restriction of algal growth by supplies of N and P – has changed in the tributaries of Chesapeake Bay following decades of reduction efforts. Toward that end, we analyzed historical data from nutrient-addition bioassay experiments and data from the Chesapeake Bay long-term water-quality monitoring program for six stations in three tidal tributaries (*i.e.*, Patuxent, Potomac, and Choptank Rivers). Classification and regression tree (CART) models were developed using concurrent collections of water-quality parameters for each bioassay monitoring location during 1990-2003, which satisfactorily predicted the bioassay-based measures of nutrient limitation (classification accuracy = 96%). Predictions from the CART models using water-quality monitoring data showed enhanced nutrient limitation over the period of 1985-2020 at four of the six stations, including the downstream station in each of these three tributaries. These results indicate detectable, long-term water-quality improvements in the tidal tributaries. Overall, this research provides a new analytical tool for detecting signs of ecosystem recovery following nutrient reductions. More broadly, the approach can be adapted to other waterbodies with long-term bioassays and water-quality data sets to detect ecosystem recovery.

1. Introduction

Eutrophication has been a major environmental issue in many coastal ecosystems around the world, primarily caused by excessive anthropogenic inputs of nitrogen (N) and phosphorus (P) (Boesch, 2019; Cloern, 2001; Kemp et al., 2009; Malone and Newton, 2020; Smith, 2003). Consequently, many restoration programs were developed to reduce watershed nutrient loads (Boesch, 2019), with the goal of lowering concentrations of N and P, among other elements, to levels that usually limit algal growth (Cloern et al., 2014; Conley, 1999; Elser et al., 2007; Hecky and Kilham, 1988). In general, inland waters (rivers and lakes) are often P-limited due to a high N:P ratio in riverine sources (e.g., Fennel and Testa 2019) and N fixation (Hecky and Kilham, 1988; Schindler, 1974), while marine waters are often N-limited due to slow N fixation and sediment release of P (Hecky and Kilham, 1988; Paerl, 2018). As transitional zones between fresh and marine waters, estuaries can exhibit more complex patterns (Cloern et al., 2014; Elser et al., 2007). For example, Chesapeake Bay, a large and complex estuary, has shown limitation of algal growth by N, P, light (L), or silicate, which vary in space and season (Conley and Malone, 1992; Fisher et al., 1999, 1992; Malone et al., 1996).

Despite efforts to reduce watershed loads of nutrients towards restoring water quality in estuaries, detecting broadly consistent estuarine effects of nutrient reductions from interannual to decadal time

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scales is complicated by spatial heterogeneity and interannual variability in the strength of the response signal to anthropogenic and natural drivers (Boynton et al., 2008; Fisher et al., 2021; Langendorf et al., 2021; Lefcheck et al., 2018; Murphy et al., 2022; Ruhl and Rybicki, 2010; Testa et al., 2019). For Chesapeake Bay, coordinated management efforts among the Bay jurisdictions began in the 1980s to reduce nutrient loads. In addition, the Bay Total Maximum Daily Load (TMDL) was established in 2010 by the U.S. Environmental Protection Agency (USEPA) to enforce nutrient reductions to improve habitat health that can fully support living resource survival, growth, and reproduction (U. S. Environmental Protection Agency, 2003, 2010). Reductions in nutrient loads are expected to lead to enhanced nutrient limitation of phytoplankton growth. However, after more than three decades of reduction efforts, it remains unclear whether nutrient limitation – an indicator of restriction of algal growth by supplies of N and P – has changed in the tidal tributaries of Chesapeake Bay. A major obstacle in characterizing such changes is the lack of consistent nutrient bioassay experiments conducted on samples of the tidal waters to assess change in nutrient limitation throughout the restoration period. Although N:P molar ratios can be computed from the rich monitoring record of estuarine nutrient concentrations to indicate the relative abundance of N and P, such ratios may incorrectly characterize the limiting nutrient and they cannot elucidate the importance of factors such as light and silicate (Chorus and Spijkerman, 2021; Ekholm, 2008; Ptacnik et al., 2010). Therefore, development of new analysis tools is needed to address this challenge and help detect signs of ecosystem recovery.

Recently, Zhang et al. (2021) developed an empirical approach using classification and regression trees (CART) to integrate temporally limited nutrient bioassay results with more comprehensive tidal water-quality monitoring samples to analyze nutrient limitation patterns in the mainstem of Chesapeake Bay. Specifically, CART was used to predict nutrient limitation for years when bioassay experiments were not available. The authors reported that the mainstem has become more limited by N during 1990-2017, which is consistent with the fact that N load from the Bay watershed has declined over the last three decades (Hirsch et al., 2010; Hyer et al., 2021; Zhang et al., 2015). The authors also noted some reduction in P-limitation in the mainstem, which is consistent with the facts that (1) flow-normalized total P load to the mainstem has increased since the mid-1990s, largely due to reduced trapping of sediment and associated P by the Lower Susquehanna River Reservoir System (Hirsch, 2012; Langland, 2015; Zhang et al., 2013; Zhang et al., 2016) and (2) flow-normalized dissolved orthophosphate load has increased in recent years (Fanelli et al., 2019).

In this work, our main objective is to apply a novel statistical approach using long-term monitoring data to investigate whether watershed nutrient load reductions have led to expanded nutrient limitation in the major tributaries of Chesapeake Bay. These tributaries are closer to terrestrial nutrient and sediment sources and are more representative of typical estuaries and their response to local management actions than the mainstem of Chesapeake Bay, which responds to geographically diverse nutrient inputs and management. We assembled historical data from nutrient bioassay experiments (1990-2003) and surface samples collected from the Chesapeake Bay Program (CBP)'s long-term, fixed-site water-quality monitoring network (1985-2020) in three tidal tributaries (Choptank, Patuxent, and Potomac Rivers) to analyze nutrient limitation at six locations (Fig. 1). Four hypotheses were tested:

- Hypothesis 1: Nutrient limitation patterns differ between these tributaries and the mainstem of the Bay, due to differences in N and P loading from their local watersheds,
- Hypothesis 2: CART can leverage concurrent tidal water-quality monitoring data to reproduce historical nutrient bioassays results,
- Hypothesis 3: Long-term changes in nutrient limitation are more prevalent in the tributaries than the mainstem due to proximity to managed nutrient sources, and

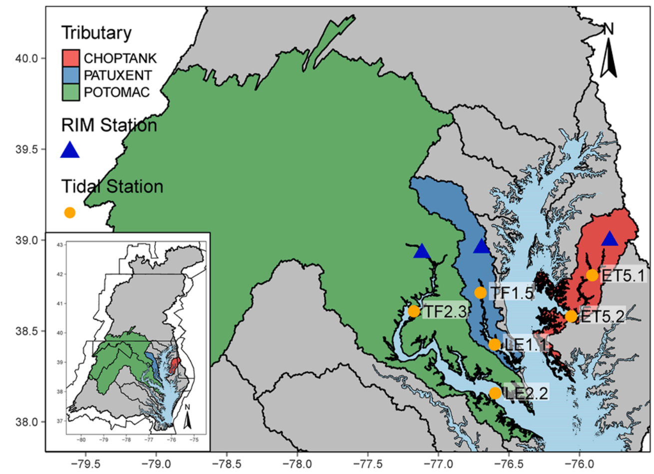

Fig. 1. Map showing the three tidal tributaries of Chesapeake Bay (*i.e.*, Choptank, Patuxent, Potomac), their River Input Monitoring (RIM) stations, and their six tidal monitoring stations analyzed in this study. The RIM station represents 16%, 40%, and 82% of the entire watershed for the Choptank, Patuxent, and Potomac Rivers, respectively. The inset map shows the locations of the three tributaries in the Chesapeake Bay watershed. See Table S1 for more details of the six monitoring stations.

- Hypothesis 4: The tidal tributaries have become more limited by nutrients following nutrient reductions.

Correspondingly, the specific goals of this work are as follows (Fig. 2):

- Goal 1: To characterize the seasonal patterns of nutrient limitation in the three tributaries,
- Goal 2: To develop CART models to predict bioassay-based nutrient limitation using tidal water-quality monitoring data in the concurrent period of 1990-2003,
- Goal 3: To apply the CART models to water-quality monitoring data in periods without bioassay data to predict nutrient limitation and explore temporal changes, and
- Goal 4: To evaluate long-term changes in nutrient limitation in each tributary in the context of long-term changes in estuarine nutrient concentrations and watershed loads.

If successful, this approach could detect long-term changes in ecosystem functioning and provide a quantitative tool that uses monitoring data to identify and evaluate ecosystem recovery following nutrient reductions in coastal ecosystems.

2. Data

We used existing data sets in this research. Here we briefly describe the data, acknowledge the data sources, and refer the reader to the original sources for more details.

2.1. Bioassays

Bioassay experiments were conducted on water samples collected at six stations in the three tributaries to characterize the response of algal growth or biomass accumulation to nutrient additions (Fisher and Gustafson, 2003, 2005; Fisher et al., 1999, 1992). These six stations, located in the Choptank (ET5.1, ET5.2), Patuxent (TF1.5, LE1.1) and Potomac (TF2.3, LE2.2) rivers (Fig. 1), had 55, 128, 61, 136, 60, and 133 bioassays performed over the period of 1990-2003, respectively (Table S1, Fig. S1). The Choptank watershed is primarily agricultural whereas the Patuxent watershed has a large fraction of developed land. The

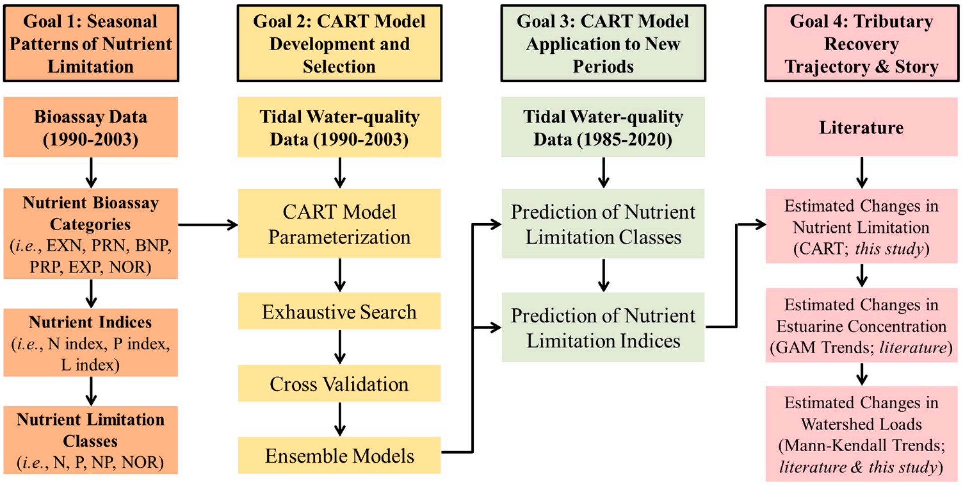

Fig. 2. Diagram showing the study data and methods for characterizing tributary nutrient limitation patterns (Goal 1), the use of bioassay data and tidal water-quality data (1990-2003) for the CART model development (Goal 2), the CART model application to predict nutrient limitation in new periods (Goal 3), and the development of tributary recovery trajectory and story (Goal 4).

Table 1
Physical, chemical, and biological parameters measured in the Chesapeake Bay tidal water-quality monitoring program which were used in this study to predict the bioassay results. For each of the listed parameters, the time scale (i.e., the time range over which each parameter is computed) is the date of sample collection.

Category	Parameter	Description	Unit
Estuarine, physical	WTEMP	estuarine water temperature	°C
	SALINITY	estuarine salinity	ppt
	SECCHI	estuarine Secchi disk depth	m
Estuarine, chemical	TN	estuarine total nitrogen	mg N L^{-1}
	DIN	estuarine dissolved inorganic N ($NO_3^- + NO_2^- + NH_4^+$)	mg N L^{-1}
	TP	estuarine total phosphorus	mg P L^{-1}
	DIP	estuarine dissolved inorganic P (PO_4^{-3})	mg P L-1
	TSS	estuarine total suspended solid	mg L^{-1}
	TN.TP.ratio	log of estuarine TN:TP ratio (molar)	unitless
	DIN.DIP.ratio	log of estuarine DIN:DIP ratio (molar)	unitless
Estuarine, biological	CHLA	estuarine chlorophyll-*a* concentration	μg L^{-1}
Watershed	Q.WS	watershed river discharge	$m^3 s^{-1}$
	TNTP.WS	log of estimated watershed TN:TP ratio (molar)	unitless
	DINDIP.WS	log of estimated watershed DIN:DIP ratio (molar)	unitless
	SS.WS	log of estimated watershed sediment load	kg d^{-1}

Potomac watershed is the second largest tributary to the Bay with several large urban centers (*e.g.*, Washington D.C.), but its upper watershed is primarily forested (Fisher et al., 2006; Irani and Claggett, 2010; Keisman et al., 2020).

For each station, nutrient limitation was characterized using three related metrics, namely, bioassay categories, nutrient indices, and nutrient limitation classes (Fig. 2). The six bioassay categories represent algal responses to nutrient additions, namely, EXN (exclusive N), PRN (primary N), BNP (balanced N and P), PRP (primary P), EXP (exclusive P), and NOR (no response), as established by Fisher et al. (1999). The bioassay categories were converted to nutrient indices ranging between 0 (no response) and 1 (response to one single resource) – see Table S2. The nutrient indices were then aggregated for each month at each station to calculate the average N index, P index, and L index representing the bioassay data period (*i.e.,* 1990-2003). Finally, the nutrient indices were used to determine the nutrient limitation classes: (1) N-limitation if N index $\geq$ 0.4; (2) P-limitation if P index $\geq$ 0.4; (3) NP limitation if both indices $\geq$ 0.4; or (4) NoR (Light limitation), otherwise. *In situ* light limitation was assumed when N and P additions did not stimulate growth greater than the controls, all of which were incubated at 50% ambient light. This scheme reduced the bioassay samples ($n = 467$) to a data set of 72 nutrient limitation classes (*i.e.,* 6 stations x 12 months). The bioassay data are archived in Zhang (2022).

2.2. *Water-quality monitoring data*

The CBP partnership maintains a long-term, tidal water-quality monitoring program with 100+ stations distributed along the mainstem and its tributaries (Tango and Batiuk, 2016; U.S. Environmental Protection Agency, 2010). Water-quality monitoring data at the six tributary stations for the period of 1985-2020 were downloaded from the CBP Water Quality Database (https://datahub.chesapeakebay.net/). See Table S1 and Fig. S2 for data coverage. Readers are referred to Zhang et al. (2021) for details of the monitoring data, including the initial screening of the relevant physical, chemical, and biological parameters as well as the pre-processing steps. See Table 1 for the parameters, which were pre-processed as follows: (1) negative and missing values were removed; (2) only data collected in the surface and above-pycnocline water-column layers were kept because they are most relevant for algal growth; (3) multiple values on the same day, if any, were averaged for each layer (*i.e.,* surface and above-pycnocline), and then the surface and above-pycnocline layer means were averaged.

In addition, watershed daily river discharges, nutrient loads, and sediment loads were computed by aggregating monitoring data from the nontidal watershed and adjoining tidal watershed (Fig. 1). For each station, the nontidal watershed is monitored at or near its fall line (*i.e.*, transition location between nontidal and tidal influence) by the U.S. Geological Survey (USGS) through its River Input Monitoring (RIM) Program (**Fig. 1**, Table S1). The RIM station represents 16%, 40%, and

82% of the entire watershed for the Choptank, Patuxent, and Potomac Rivers, respectively. Loads at the station (called "RIM loads") are reported by the USGS using the Weighted Regressions on Time, Discharge, and Season (WRTDS) method (Mason et al., 2021). The watershed adjacent to tidal waters is not monitored and its loads (called "below-RIM" loads) and river flows were estimated from the CBP Phase 6 Watershed Model (Chesapeake Bay Program, 2020) using the tidal segments above each station (see Table S1). For example, the watershed load for Patuxent TF1.5 is the sum of its RIM load (USGS station 01594440) and below-RIM load (tidal segments WBRTF and PAXTF); the watershed load for Patuxent LE1.1 is the sum of its RIM load (USGS station 01594440) and below-RIM load (tidal segments WBRTF, PAXTF, PAXOH, and PAXMH). Like the bioassay data, the water-quality monitoring data are archived in Zhang (2022).

3. Methods

3.1. CART

CART models were developed to relate the nutrient bioassay categories in Table S2 ("response variable") to a set of initially selected water-quality parameters in Table 1 ("explanatory variables") for the period of 1990-2003 for each tributary station (Fig. 2). CART was chosen because it has previously been used to model nutrient limitation in the mainstem of Chesapeake Bay (Zhang et al., 2021). In brief, CART splits the full data, which are contained in the "top node" on the tree, sequentially into subgroups ("intermediate nodes"), and stops at the bottom of the tree ("terminal nodes") (Breiman, 1984). At every node except the terminal nodes, CART evaluates all possible split points for each explanatory variable and selects the variable and split point that can lead to two most dissimilar subgroups. Ideally, CART trees should not be too large (over-fit) or too small (under-fit) and should have informative splitting rules and pure terminal nodes (Breiman, 1984; Loh, 2014). CART can incorporate multiple explanatory variables and thus utilize information beyond nutrient concentrations (*e.g.*, water temperature). In addition, CART allows flexibility in relating the response variable to the explanatory variables, including multiple cutoffs and interactions (*e.g.*, a variable can appear multiple times on the tree with different splitting thresholds). Moreover, our prior work (Zhang et al., 2021) showed similar classification performance between CART and other machine learning methods (i.e., random forest and boosted trees).

3.2. Exhaustive search of candidate variables

To determine the optimal CART models, an exhaustive search algorithm was developed in R statistical software to evaluate all possible combinations of the candidate variables for predicting nutrient bioassay results at each tributary station (Fig. 2). This new algorithm searches for a global optimum, because the model form can start with any explanatory variable and incrementally add any of the remaining variables to improve prediction. For parsimony, this algorithm was set to allow no more than six variables. (Note that some model forms had fewer than six variables because the algorithm sampled the variables with replacement.)

CART models were implemented in the "rpart" package (Therneau and Atkinson, 2019) with the following parameterization: $minsplit = 2$, $maxdepth = 5$, $cp = 0.01$. In the exhaustive search, all candidate model forms were evaluated using the leave-one-out cross validation (LOOCV), which provides a fair assessment of the model's classification accuracy and gives insights on how well the model generalizes to a new data set. In each iteration, one data record was left out and treated as new data. The remaining data records were used to train the model, which was then used to make the prediction for the left-out data. For each model, the overall accuracy was calculated as the weighted average of the category-based accuracies.

3.3. Ensemble models

For each tributary station, three model forms with the highest LOOCV accuracy were retained for the next step. In general, the three CART model forms selected for bioassay prediction at each station have different trees and different best-performing bioassay categories based on their confusion matrices – see Table S3. Therefore, an ensemble model was developed for each tributary station to combine the strength of the three model forms (Fig. 2). This approach compares predictions from each of the three CART models and selects the predicted class that has the highest probability. For cases where model predictions diverge, the prediction with the highest probability is selected. The advantage of such an ensemble model approach has been demonstrated in our prior analysis of the mainstem nutrient limitation (Zhang et al., 2021).

Like the observed bioassay categories, predictions of bioassay categories from the ensemble model for each tributary station were converted to nutrient indices, which were then aggregated to long-term (1990-2003) average nutrient indices for each calendar month. Finally, the average nutrient indices were used to determine the nutrient limitation classes, as described in Section 2.1. The CART-predicted nutrient limitation classes were compared with the bioassay-based classes to quantify the performance of the CART ensemble models. The R code for the CART model analysis is archived in Zhang (2022).

3.4. Model application

To explore potential temporal changes in nutrient limitation, the ensemble model for each of the six tributary stations was applied to new periods without bioassay data to predict nutrient limitation (Fig. 2). First, predictions were made for two decadal periods – *i.e.,* 1985–1994 and 2011–2020, and the resulting decadal average nutrient limitation classes were compared to assess temporal changes. Note that all available water-quality monitoring data (*i.e.*, all months and years) were used in this analysis, as opposed to the model development step, where only those paired with the bioassay samples were used (Table S1).

An additional means of quantifying changes in nutrient limitation, new relative to Zhang et al. (2021), was to apply the ensemble models to three-year running windows over the period of 1985-2020 to compute average nutrient indices for each window and then to compute the Mann-Kendall trends of the nutrient indices (Kendall, 1975; Sen, 1968). This approach has three advantages: (1) it avoids the subjectivity in choosing the thresholds (e.g., 0.4) when converting nutrient indices to nutrient limitation classes (see Section 2.1), (2) it avoids the choice of two temporal periods and fully utilizes the entire period of data for detecting changes, and (3) it better distinguishes nutrient indices that belong to the same nutrient limitation classes (*e.g.*, two N indices, 0.6 and 0.7, both correspond to N-limitation, but the latter indicates a stronger N-limitation).

3.5. Tributary stories

To provide a portrait of long-term changes for each of the three tributaries, the CART-estimated changes in nutrient limitation were evaluated in the context of estimated changes in monitored estuarine nutrient concentrations and estimated changes in watershed loads (Fig. 2). For estuarine concentrations (TN, TP, and TSS), long-term trends were calculated by first fitting Generalized Additive Models (GAMs) to the observed data for the period of 1985-2020 with explanatory variables including date, day of year, and upstream river flow. The fitted GAM models were then used to estimate what the estuarine concentrations would have been if freshwater flow had been average for the entire period (*i.e.*, flow-adjusted concentrations). The percent change of these flow-adjusted concentrations between the start and end of the period of interest is the GAM trend (Murphy et al., 2019). For watershed loads (TN, TP, DIN, DIP, and SS), the RIM load and below-RIM load were aggregated for each monitoring station (see Section 2.2), and the long-term trends were calculated using the Mann-Kendall Test (Kendall, 1975; Sen, 1968). Furthermore, watershed loads were divided by river discharges to obtain the so-called flow-weighted concentrations (FWCs), which can better reveal temporal trends in the riverine inputs by constraining the effect of interannual variability in river discharges (Zhang et al., 2022a).

4. Results

4.1. Seasonal patterns of tributary nutrient limitation (Goal 1)

Historical nutrient bioassays (1990-2003) provide a summary of the seasonal patterns of nutrient limitation in each of the three tidal tributaries (left side of each graph in Fig. 3). In the Choptank River at the upstream station (ET5.1), NoR is prevalent (*i.e.,* 9 out of 12 months) with the exception that the warmer months (July – September) are classified as N-limitation. At the downstream station (ET5.2), NoR is less prevalent (*i.e.,* 2 out of 12 months), and most months show N-limitation, P-limitation, or both (with N-limitation most common).

In the Patuxent River at the upstream station (TF1.5), NoR is prevalent in the early months of the year (January – May), whereas the later months are classified as N-limitation, P-limitation, or both (with N-limitation most common). At the downstream station (LE1.1), NoR is absent and most months are classified as N-limitation (*i.e.,* 10 out of 12 months).

In the Potomac River at the upstream station (TF2.3), NoR is prevalent (*i.e.,* 10 out of 12 months) except that August and September are classified as P-limitation. At the downstream station (LE2.2), all four types of nutrient limitation are present, generally with NoR in the winter months, P-limitation in the spring months, and N-limitation in the summer-fall months.

Overall, the upstream stations in these tributaries are strongly dominated by NoR, followed by N-limitation during warm seasons, with rare occurrence of P- and NP-limitation. In contrast, the downstream stations show limited presence of NoR, moderately more P- and NP-limitation, and much more N-limitation.

4.2. Development of CART models for modeling tributary nutrient limitation (Goal 2)

The exhaustive search algorithm identified three CART model forms for bioassay prediction at each tributary station. The ensemble model for each station combined the strength of the three selected models by preserving the highest classification accuracy for each applicable bioassay category (Table 2). For example, the ensemble model for ET5.1 has an accuracy of 82% and 96% for EXN ($n = 11$) and NOR ($n = 24$), respectively, and an overall accuracy of 86% for all bioassay categories ($n = 43$). For all six tributary stations, the ensemble models have an overall accuracy between 69% and 88% with a median of 82%.

The ensemble models were further evaluated for nutrient limitation classes in the 1990-2003 period, which were converted from the bioassay categories for each month at each station (right side of each graph in Fig. 3). Of the 72 cases (*i.e.,* 12 months x 6 stations), only three showed mismatches between the CART-based and the bioassay-based nutrient limitation classes, including one month at ET5.2 (Choptank), one month at LE1.1 (Patuxent), and one month at T2.3 (Potomac).

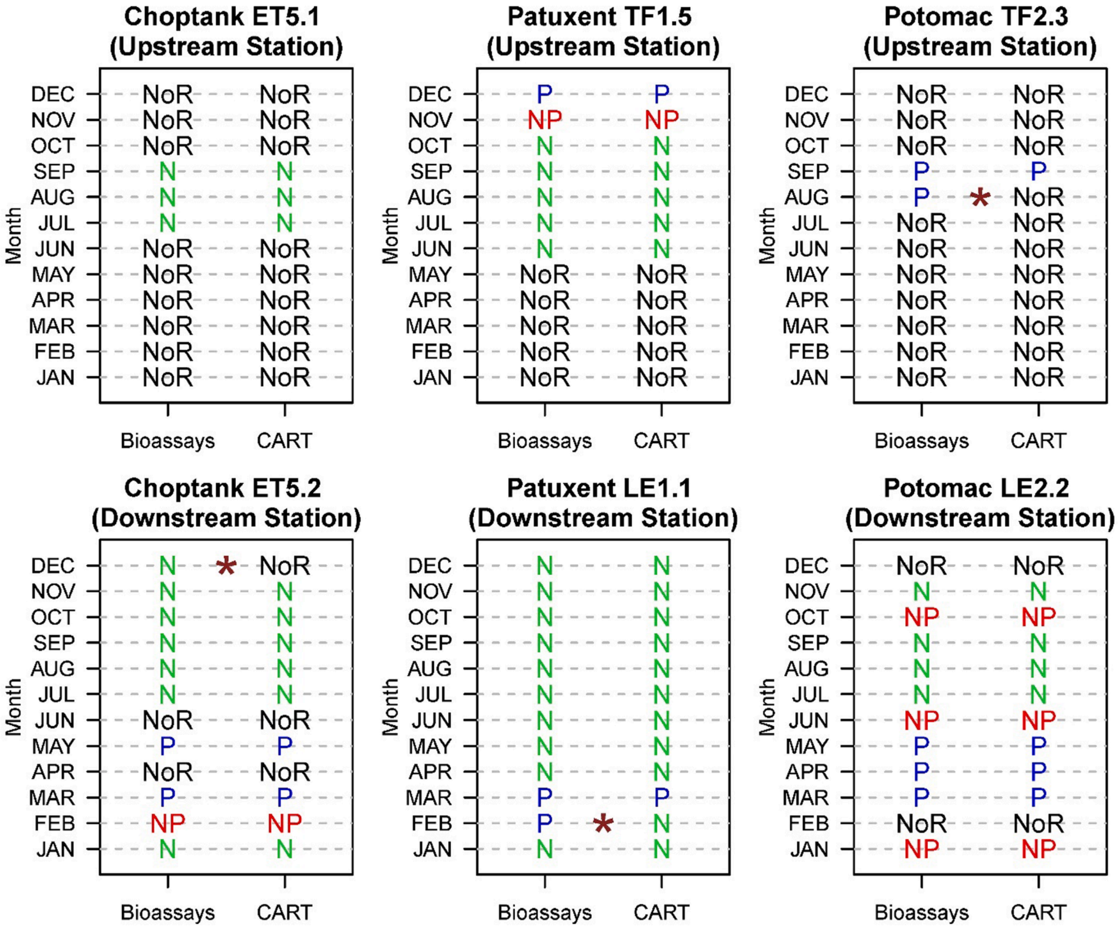

Fig. 3. Nutrient limitation diagrams for the six monitoring stations, comparing bioassay-based nutrient limitation classes and CART-based classes. The CART-predicted classes were based on the ensemble approach that incorporated the three candidate models specific to each station. Brown stars indicate mismatches; count = 3 (out of 72).

Table 2
Summary of the sample count and model accuracy by bioassay category as well as the selected variables for CART models for each monitoring station.

River	Station	Sample count and model accuracy (in percent) by bioassay category[1]	BNP	EXN	EXP	NOR	PRN	PRP	Overall[2]	Explanatory variables selected by the ensemble model approach[3]
Choptank River	ET5.1	Sample No.	1	11	0	24	3	4	43	DIN, DIP, DINDIP.ratio, SECCHI, Q.WS, DINDIP.WS, SS.WS
		Accuracy	-	82	-	96	67	75	86	
	ET5.2	Sample No.	9	14	2	21	42	19	107	DIN, DIP, DINDIP.ratio, TP, WTEMP, SALINITY, DINDIP.WS
		Accuracy	33	50	-	76	86	74	71	
Patuxent River	TF1.5	Sample No.	1	17	2	25	4	0	49	DIN, DIP, TN, TP, TSS, Q.WS, DINDIP.WS, TNTP.WS
		Accuracy	-	100	-	92	50	-	86	
	LE1.1	Sample No.	15	19	3	15	59	6	117	DIN, DIP, DINDIP.ratio, TN, TP, TNTP.ratio, TSS, CHLA, WTEMP, SALINITY
		Accuracy	53	79	33	80	90	33	78	
Potomac River	TF2.3	Sample No.	1	0	6	25	1	10	43	DIN, DIP, DINDIP.ratio, TSS, CHLA, WTEMP, SECCHI, DINDIP.WS
		Accuracy	-	-	100	96	-	80	88	
	LE2.2	Sample No.	16	8	7	12	39	26	108	DIN, DIP, TP, TSS, WTEMP, SECCHI, Q.WS, DINDIP.WS
		Accuracy	50	50	57	33	85	81	69	

[1] The model accuracy for each bioassay category was determined using the leave-one-out cross validation (LOOCV). Classification accuracy is provided for bioassay categories with at least three samples.

[2] The "Overall" accuracy was calculated as the weighted average of the category-based accuracies.

[3] For details of the ensemble models, see Table S3.

Therefore, the station-specific CART models satisfactorily reproduced the bioassay-based nutrient limitation classes (classification accuracy = [1- 3/72] * 100% = 96%). In comparison, our CART models published for the mainstem of Chesapeake Bay (Zhang et al., 2021), when applied to these tributary stations, resulted in 36 mismatches (accuracy = [1 - 36/72] * 100% = 50%) with the bioassay-based nutrient limitation classes (Fig. S3).

Our ensemble models for the tributary stations have selected different combinations of explanatory variables, suggesting different controls on nutrient limitation (Table 2). For example, the two Patuxent stations are both affected by DIN and DIP, but only the upstream station (TF1.5) is also affected by watershed-relevant variables, *i.e.*, river discharge (Q.WS), the ratio of DIN to DIP (DINDIP.WS), and the ratio of TN to TP (TNTP.WS). Among the six stations, the most common explanatory variables selected by CART are DIN, DIP ($n = 6$); DINDIP.WS ($n = 5$); and DINDIP.ratio, WTEMP, TSS and TP ($n = 4$). Specifically, DIN, DIP, and DINDIP.WS are influential at all three upstream stations, whereas DIN, DIP, TP, and WTEMP are influential at all three downstream stations.

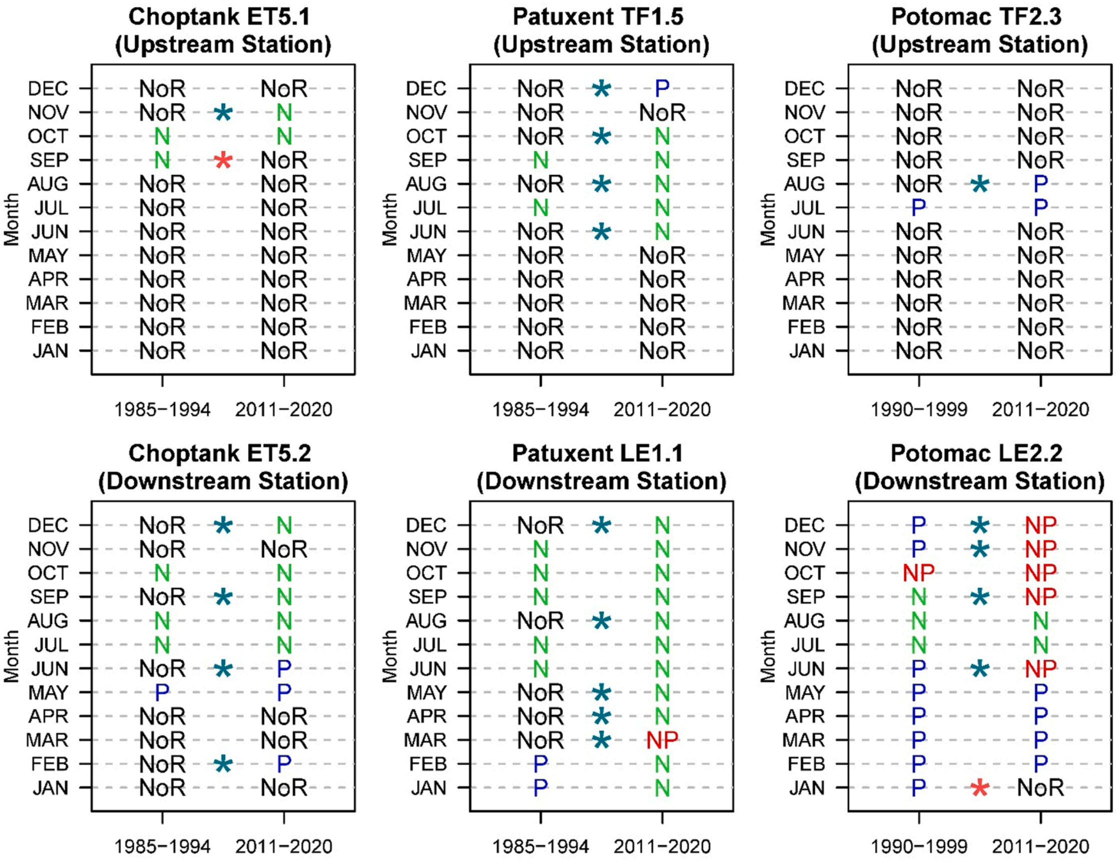

Fig. 4. Nutrient limitation diagrams for the six monitoring stations, comparing CART predictions for two decadal periods: 1985-1994 *vs.* 2011-2020. For the Potomac stations, the period of 1990-1999 was used due to data unavailability prior to 1990. These predictions were based on the ensemble approach that incorporated the three candidate models specific to each station. Cyan stars indicate enhanced nutrient limitation while red stars indicate weakened nutrient limitation.

Table 3

Mann-Kendall trends in the estimated monthly N index, P index, and L index for the six monitoring stations for the period of 1985-2020. Green cells indicate enhanced nutrient limitation (or weakened light limitation; $p < 0.1$), whereas red cells indicate weakened nutrient limitation (or enhanced light limitation; $p < 0.1$). "*ns*" indicates non-significant trends ($p > 0.1$).

	Choptank ET5.1 (Upstream Station)			Patuxent TF1.5 (Upstream Station)			Potomac TF2.3 (Upstream Station)		
Month	N index	P index	L index	N index	P index	L index	N index	P index	L index
Count$_{\text{Recovery}}$	4	2	2	5	2	5	0	1	1
Count$_{\text{Degradation}}$	2	2	1	0	0	0	0	1	1
DEC	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
NOV	0.009	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
OCT	*ns*	*ns*	*ns*	0.015	0.011	-0.025	*ns*	*ns*	*ns*
SEP	-0.006	*ns*	*ns*	0.015	0.004	-0.015	*ns*	-0.002	0.006
AUG	*ns*	0.002	*ns*	0.019	*ns*	-0.018	*ns*	0.013	-0.011
JUL	-0.009	-0.002	0.011	0.010	*ns*	-0.014	*ns*	*ns*	*ns*
JUN	*ns*	*ns*	*ns*	0.006	*ns*	-0.010	*ns*	*ns*	*ns*
MAY	0.003	0.005	-0.009	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
APR	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
MAR	*ns*	-0.002	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
FEB	0.009	*ns*	-0.012	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
JAN	0.008	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
	Choptank ET5.2 (Downstream Station)			Patuxent LE1.1 (Downstream Station)			Potomac LE2.2 (Downstream Station)		
Month	N index	P index	L index	N index	P index	L index	N index	P index	L index
Count$_{\text{Recovery}}$	4	7	6	10	5	6	5	2	0
Count$_{\text{Degradation}}$	0	0	0	0	2	0	1	3	0
DEC	*ns*	*ns*	*ns*	0.015	0.004	-0.014	*ns*	*ns*	*ns*
NOV	*ns*	0.004	*ns*	*ns*	*ns*	*ns*	0.006	*ns*	*ns*
OCT	*ns*	0.006	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*	*ns*
SEP	*ns*	*ns*	*ns*	0.013	*ns*	-0.008	*ns*	*ns*	*ns*
AUG	0.012	*ns*	-0.010	0.020	0.004	-0.014	-0.003	0.003	*ns*
JUL	*ns*	0.008	*ns*	0.004	*ns*	*ns*	*ns*	*ns*	*ns*
JUN	*ns*	0.017	-0.009	0.003	0.002	*ns*	0.005	*ns*	*ns*
MAY	0.005	0.012	-0.017	0.011	*ns*	-0.007	0.003	-0.003	*ns*
APR	0.006	0.007	-0.012	0.019	0.005	-0.023	0.010	-0.004	*ns*
MAR	0.007	*ns*	-0.012	0.009	0.013	-0.015	0.007	0.006	*ns*
FEB	*ns*	0.009	-0.015	0.020	-0.013	*ns*	*ns*	-0.009	*ns*
JAN	*ns*	*ns*	*ns*	0.014	-0.010	*ns*	*ns*	*ns*	*ns*

4.3. Application of CART models for quantifying temporal changes in nutrient limitation (Goal 3)

CART predictions of nutrient limitation for the six tributary stations showed temporal changes between the two decadal periods: 1985-1994 *vs.* 2011-2020 (Fig. 4). Of the 72 station-month pairs, enhanced nutrient limitation occurred in 6 pairs at the upstream stations and 13 pairs at the downstream stations. Of these pairs, 15 involved NoR changing to N-, P-,

Table 4

Summary of CART-predicted nutrient limitation trajectories as well as significant trends in estuarine nutrient annual concentrations and watershed nutrient annual loads for the six monitoring stations for the period of 1985-2020. Increases in estuarine concentrations, watershed loads, or watershed flow-weighted concentrations ("↑") are indicative of degradations ($p < 0.1$), whereas decreases in these variables ("↓") indicate recoveries ($p < 0.1$). "*ns*" indicates non-significant trends ($p > 0.1$).

River	Station	Nutrient Limitation Trajectory	Estuarine Flow-adjusted Concentration[1]			Watershed True-condition Load[1]					Watershed Flow-weighted Concentration[1]				
			TN	TP	TSS[2]	TN	DIN	TP	DIP	SS	TN	DIN	TP	DIP	SS
Choptank River	ET5.1	Mixed Changes	↑	↓	↓	*ns*	*ns*	*ns*	*ns*	*ns*	↓	↓	↓	↓	*ns*
	ET5.2	Recovery	↓	↓	↓	*ns*	*ns*	*ns*	↓	*ns*	↓	↓	↓	↓	*ns*
Patuxent River	TF1.5	Recovery	↓	↓	↑	↓	↓	*ns*	↓	↑	↓	↓	↓	↓	↑
	LE1.1	Recovery	↓	↓	↓	↓	↓	*ns*	↓	↑	↓	↓	↓	↓	*ns*
Potomac River	TF2.3	Limited Change	↓	↓	↓	↓	↓	*ns*	↓	*ns*	↓	↓	*ns*	↓	*ns*
	LE2.2	Recovery	↓	↓	*ns*	↓	↓	*ns*	↓	*ns*	↓	↓	*ns*	↓	*ns*

[1] For details on data and trend approaches, see Sections 2.2 and 3.5.

[2] For TSS estuarine concentration only, the long-term trend was analyzed for the period of 1999-2020 because of a change in laboratory that occurred in 1998.

or NP-limitation and 4 involved N- or P-limitation changing to NP-limitation. Only two station-month pairs showed weakened nutrient limitation, one at Choptank ET5.1 (N changing to NoR) and the other at Potomac LE2.2 (P changing to NoR). Overall, four of these six stations (*i.e.*, all three downstream stations plus Patuxent TF1.5) were predicted by the ensemble models to have clearly become more limited by nutrients.

Mann-Kendall trends quantified for the CART-estimated monthly nutrient indices over the period of 1985-2020 (Table 3) are generally consistent with the comparisons between the two decadal periods (Fig. 4). Overall, four of the six stations (*i.e.*, all three downstream stations plus Patuxent TF1.5) were predicted by the ensemble models to show a preponderance of increasing N or P indices (*i.e.*, enhanced nutrient limitation). Collectively, these four stations showed 24 months of enhanced N-limitation, 16 months of enhanced P-limitation, and 17 months of weakened L-limitation. By contrast, Choptank ET5.1 showed mixed changes in nutrient indices and TF2.3 (Potomac River) showed limited change.

4.4. *Development of tributary stories on long-term changes (Goal 4)*

Here we provide a portrait of long-term changes in the three tributaries by linking the estimated changes in nutrient limitation with the estimated changes in monitored estuarine nutrient concentrations and estimated changes in watershed loads and their flow-weighted concentrations (FWCs) (Table 4). In the Choptank River, ET5.1 showed *mixed changes*. The lack of a sustained reduction in N-limitation is consistent with the statistically significant increase in estuarine TN concentration at this station. Although FWCs of TN, DIN, TP, and DIP showed

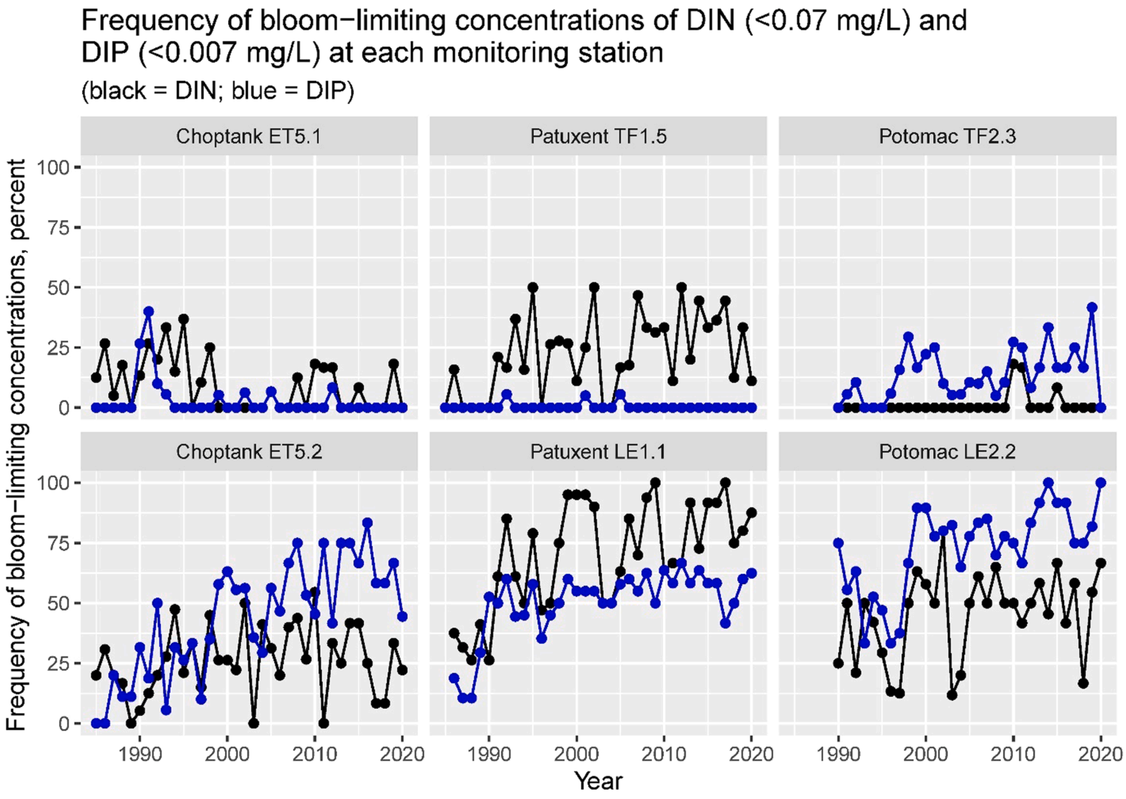

Fig. 5. Percentages of bloom-limiting concentrations of DIN (< 0.07 mg/L) and DIP (< 0.007 mg/L) for the six monitoring stations in each year over the period of 1985-2020.

statistically significant declines, their watershed loads and SS loads showed non-significant trends. In contrast with ET5.1, ET5.2 showed a *recovery trajectory* – *i.e.,* more N- and P-limitation and less NoR. These changes are consistent with the statistically significant declines in estuarine TN, TP, and TSS concentrations at this station. TN, DIN, TP, and DIP all showed statistically significant declines in terms of FWC, although only DIP showed a statistically significant decline in terms of load.

In the Patuxent River, TF1.5 and LE1.1 both showed *recovery trajectories* – *i.e.,* more N- and P-limitation and less NoR. These changes are consistent with the statistically significant declines in estuarine TN and TP concentrations at these stations. They are also consistent with the statistically significant declines in TN, DIN, and DIP loads and significant declines in TN, DIN, TP, and DIP FWCs.

In the Potomac River, TF2.3 showed *limited change* in nutrient limitation. This is not consistent with the statistically significant declines in estuarine TN, TP, and TSS concentrations at this station, or the statistically significant declines in TN, DIN, and DIP loads and their FWCs. In contrast with TF2.3, LE2.2 showed a *recovery trajectory* – *i.e.,* more N- and P-limitation and less NoR. These changes are consistent with the statistically significant declines in estuarine TN and TP concentrations at this station. They are also consistent with the statistically significant declines in TN, DIN, and DIP loads and their FWCs.

5. Discussion

5.1. Hypothesis 1: Nutrient limitation patterns differ between these tributaries and the mainstem of the Bay, due to differences in N and P loading from their local watersheds

The bioassay results show different seasonal and spatial patterns of nutrient limitation in the tributaries (Fig. 3). The spatial contrast (predominant NoR upstream, N- and P- limitation downstream) is consistent with high turbidity and/or high ambient nutrient concentrations at upstream stations in close proximity to watershed loads and in contrast, lower turbidity and lower nutrient concentrations at downstream stations, indicating high rates of sedimentation and nutrient assimilation (*e.g.,* plant uptake, denitrification) that limit seaward nutrient fluxes (Buchanan, 2020; Fisher et al., 2021; Noe et al., 2020; Testa et al., 2013). These results are consistent with the tidal monitoring data, which show small proportions of bloom-limiting concentrations of DIN and DIP at the upstream stations but much larger proportions of bloom-limiting concentrations at the downstream stations (Fig. 5). This spatial contrast in nutrient limitation in the tributaries is consistent with the mainstem of the Bay (Fisher et al., 1999; Kemp et al., 2005; Zhang et al., 2021). Although the spatial scales differ between the tributaries and the mainstem, they consistently show NoR in the upper regions due to conditions of nutrient saturation and high turbidity, in contrast with more frequent nutrient limitation in the lower (more saline) regions. Thus, our results do not support *Hypothesis* 1.

5.2. Hypothesis 2: CART can leverage concurrent tidal water-quality monitoring data to reproduce historical nutrient bioassays results

The station-specific CART models satisfactorily reproduced the bioassay-based nutrient limitation classes using tidal water-quality monitoring data (classification accuracy = 96%) (Fig. 3). By contrast, the CART models previously published for the mainstem (Zhang et al., 2021) failed to adequately reproduce nutrient limitation in the tributaries (accuracy = 50%) (Fig. S3). Therefore, we recommend the development of station-specific CART models for the tidal tributaries to capture unique patterns and drivers of nutrient limitation. These results support *Hypothesis 2* and show that CART is an accurate estimation tool for characterizing nutrient limitation for times without bioassay measurements, thereby greatly expanding the temporal scales of such assessment. In this regard, bioassay measurements in other major tributaries (e.g., James, Rappahannock, and York) would be helpful for development of CART models. Depending on the availability of resources, sampling monthly or seasonally for several years at each selected location to capture different conditions could help with model development.

Our ensemble models for the tributary stations have selected different combinations of explanatory variables (Table 2), suggesting different controls on nutrient limitation. Among the six stations, the most common explanatory variables selected by CART are DIN, DIP, DINDIP.WS, DINDIP.ratio, WTEMP, TSS, and TP. Specifically, DIN, DIP, and DINDIP.WS are influential at all three upstream stations, whereas DIN, DIP, TP, and WTEMP are influential at all three downstream stations. The selected variables show that phytoplankton growth can be affected by estuarine chemical, biological, and physical conditions as well as watershed inputs. Notably, DIN and DIP concentrations are selected by the ensemble models of all six tributary stations, which represent the amount of nutrients available for phytoplankton growth at a given moment. When light (TSS) and other factors are not controlling phytoplankton growth, nutrient concentrations determine how much of the light-induced potential of phytoplankton for photosynthesis and growth can be realized (Buchanan, 2020; Fisher et al., 1999, 1992). Concentrations above bloom-limiting thresholds (*i.e.,* >0.07 mg l^{-1} for DIN; >0.007 mg l^{-1} for DIP) are associated with a lack of response to nutrient additions (Fisher and Gustafson, 2003), and riverine concentrations typically exceed these thresholds by 2-3 orders of magnitude (Fisher et al., 2021). In addition, TSS increases light attenuation and leads to enhanced light limitation *in situ* (Bilotta and Brazier, 2008; Buchanan, 2020; Cerco et al., 2013; Testa et al., 2019; Turner et al., 2021). Beyond these chemical parameters, WTEMP represents the integrated effect of seasonal shifts due to temperature effects on maximum phytoplankton growth rates, day length and daylight available for photosynthesis, as well as seasonal changes in mixing, light field, and plankton community composition (Droop, 1983; Fisher et al., 1999, 1992; Kemp et al., 2005; Malone et al., 1996). The local variations in these potential controls on phytoplankton growth can lead to spatial and temporal variations in light and nutrient limitation in the tributaries, and our CART models reveal these local controls.

Lastly, we note that the performance of the tributary CART models is inevitably constrained by the precision of bioassay experiments, the sample size of each bioassay category, and the inherent variability in water samples (Fisher and Gustafson, 2003,2005; Fisher et al., 1999, 1992). Moreover, a key assumption of CART is the stationarity in the derived relationships. This caveat can inadvertently force predictions for new periods to strictly follow a particular splitting rule of the classification tree to fall into a fixed terminal node (class), regardless of any changes in explanatory variables irrelevant to that splitting rule (Zhang et al., 2021). In this regard, additional bioassays can be useful for validating and/or re-calibrating these CART models following known changes in local watershed inputs or developing new CART models in tributaries where no bioassays have yet been done. Furthermore, the tidal water-quality monitoring data can provide some level of validation. For example, the CART inference on the nutrient limitation conditions and changes is generally supported by the patterns of estuarine DIN and DIP concentrations (Fig. 5).

5.3. Hypothesis 3: Long-term changes in nutrient limitation are more prevalent in the tributaries than the mainstem due to proximity to managed nutrient sources

Our results also support *Hypothesis 3*. Predictions from the CART models showed long-term recovery trajectories at four of the six tributary stations, including all three downstream stations, which indicate long-term water-quality improvements (Table 3, Fig. 4). In contrast with nutrient limitation in the mainstem of the Bay (Zhang et al., 2021), nutrient limitation in these tributaries have had more occurrences of change: specifically, enhanced nutrient limitation is observed with 19 station-month pairs in the decadal comparison (Fig. 4). The mainstem of the Bay is influenced by nutrient inputs from all major tributaries, among which the Susquehanna River is the largest in terms of freshwater and nutrient inputs (Hagy et al., 2004; Murphy et al., 2011; Zhang et al., 2015). As reported previously, the mainstem has become more limited by N in the more recent period, consistent with the long-term reduction in N load (Hirsch et al., 2010; Hyer et al., 2021; Zhang et al., 2015). However, the mainstem showed some reduction in P-limitation, consistent with increases in TP and DIP loads (Fanelli et al., 2019; Hirsch, 2012; Zhang et al., 2013, 2016). Compared to the mainstem, the three tributaries analyzed in this study, especially their upstream stations, are less influenced by the Susquehanna River. In addition, these tributaries are embedded within the landscape and closer to watershed sources than the mainstem. These configurations can result in generally stronger responses in the tributaries to nutrient reductions than the mainstem, as shown in the CART-estimated changes in nutrient limitation. Furthermore, each of the three tributaries analyzed include major wastewater treatment plants [WWTPs] that were upgraded during the study period (Boynton et al., 2008; Fisher et al., 2021; Lyerly et al., 2014; Ruhl and Rybicki, 2010). In addition to local nutrient sources, these tributaries are also connected with the mainstem of the Bay, and the generally stronger responses at the downstream stations than the upstream stations may suggest that these tributaries are also positively influenced by the water-quality improvement in the mainstem that reflects watershed-wide nutrient reductions.

5.4. *Hypothesis 4: The tidal tributaries have become more limited by nutrients following nutrient reductions*

Our results also provide evidence that supports *Hypothesis 4* (Table 4). The long-term recovery trajectories observed in these tributaries align well with other documented water-quality improvements that have been linked to management efforts toward nutrient reductions (Boynton et al., 2008, 2013; Fisher et al., 2021; Jones, 2020; Lefcheck et al., 2018; Murphy et al., 2022, 2011; Ruhl and Rybicki, 2010; Testa et al., 2019; Zhang et al., 2021, 2018). These efforts have been pursued through the implementation of wastewater, atmospheric, agricultural, and urban stormwater source controls to address the reduction goals outlined in the Chesapeake Bay Partnership Agreements (Chesapeake Bay Partnership, 1983; Chesapeake Executive Council, 1987, 2000, 2014) and the Bay TMDL (U.S. Environmental Protection Agency, 2010).

In the Choptank River, the mixed trends at ET5.1 indicate the lack of sufficient reduction in nutrient loads, with N believed to be affected by (a) groundwater legacies due to accumulations of historical agricultural inputs (Chang et al., 2021; Hirsch et al., 2010) and (b) a long-term increase in river discharge (data not shown). In addition, concentrations at ET5.1 have not declined to sufficiently low levels to trigger nutrient limitation, as demonstrated by the small proportions of bloom-limiting concentrations of DIN and DIP (Fig. 5). Therefore, stronger reductions in nutrient loads are needed for making progress at ET5.1. By contrast, the recovery trajectory at ET5.2 highlights the importance of nutrient reductions from the below-RIM watershed. Since nonpoint sources in the Choptank have shown a statistically significant increase (Fisher et al., 2021), this recovery can be attributed to point source reductions, as achieved by the P detergent ban and technology upgrades at WWTPs, especially the Cambridge and Easton WWTPs (Fisher et al., 2021; Lyerly et al., 2014).

In the Patuxent River, both stations showed enhanced nutrient limitation, consistent with reductions in nutrient loads from the watershed. For N, the reduction reflects a combination of reductions in RIM load and below-RIM load driven by biological nutrient removal at major WWTPs (Boynton et al., 2008; Lyerly et al., 2014) and reductions of atmospheric deposition (Eshleman et al., 2013; Lyerly et al., 2014). Like the Choptank River, the Patuxent River has benefited from P reduction from point sources, owing to the P detergent ban and technology upgrades at WWTPs (Boynton et al., 2008; Lyerly et al., 2014).

In the Potomac River, TF2.3 showed a lack of change in nutrient limitation despite the significant declines in estuarine concentrations and watershed loads. Given that TF2.3 has been dominated by NoR (Fig. 3), it is likely that light availability is still the primary limiting factor to phytoplankton growth, which can hinder the responses of phytoplankton to nutrient reductions. Another explanation is that nutrient reductions from the watershed have not resulted in sufficiently low estuarine concentrations to trigger nutrient limitation, as demonstrated by the small proportions of bloom-limiting concentrations of DIN and DIP (Fig. 5). In addition to external sources, internally accumulated nutrients may play a role and delay the response of water-column nutrient concentrations to watershed load reductions (Jones, 2020). By contrast, the recovery trajectory at LE2.2 highlights the importance of instream processing and/or nutrient reductions from the below-RIM watershed, especially in the areas between the two stations. The reduction in N loads reflects a combination of reductions in RIM load and below-RIM load with some of the major reductions attributed to point sources and atmospheric deposition. While the atmospheric deposition reduction is linked to the Clean Air Act (Eshleman et al., 2013; Lyerly et al., 2014), the point source reduction is attributed to biological nutrient removal at major WWTPs in the D.C.-metropolitan region, especially the Blue Plains Advanced WWTP, the largest of its kind in the Bay watershed (Lyerly et al., 2014; Ruhl and Rybicki, 2010). The reduction in P loads is primarily due to the detergent ban and technology upgrades at WWTPs (Lyerly et al., 2014; Ruhl and Rybicki, 2010).

5.5. *Summary and management implications*

Our results demonstrate long-term recovery trajectories in the tidal tributaries to Chesapeake Bay, which have management implications for Chesapeake Bay and other coastal ecosystems. The first main message is that management efforts focused on dual nutrient reductions have resulted in long-term water-quality improvements in the tidal tributaries of Chesapeake Bay. This conclusion inferred from our CART predictions is consistent with studies on other waterbodies. For example, monitoring and bioassay data collected in the James River, which is the third largest tributary to Chesapeake Bay, showed that nutrient reductions have led to declined nutrient concentrations and enhanced nutrient limitation (Wood and Bukaveckas, 2013). A study on the continental shelf of the northern Gulf of Mexico predicted an expansion of P-limitation following reductions in DIP concentrations in the Mississippi and Atchafalaya Rivers (Laurent et al., 2012), and their results compare well with observations. In the Neuse River Estuary, the reduction of P load (due to a P detergent ban and improved wastewater removal) resulted in a decrease in upstream freshwater phytoplankton and nuisance algal blooms (Paerl et al., 2004). In a Danish study, coastal waters showed clear signs of improvement following reductions of N and P loads since 1990, including significant declines in nutrient concentrations, stronger potential P limitation in the 1990s, and stronger potential N limitation thereafter (Riemann et al., 2015). In the River Loire (France), total phytoplankton and cyanobacteria biomass declined significantly since 1991 due to the reduction of riverine P (Minaudo et al., 2021). Similarly, an Irish study on 18 river systems reported significant reductions of P load from 15 watersheds, which led to parallel improvements in estuarine water in eight downstream systems with significant decreases in estuarine P concentration (Longphuirt et al., 2016). In the inner Oslofjorden (Norway), declined P concentration has contributed to the decrease in chlorophyll-a from 1980s to 1990s (Lundsør et al., 2020). In the Toyama Bay (Japan), 25 years of reduction of P load, especially from WWTPs, has led to significant reductions of riverine loads and enhanced P limitation in coastal waters (Katazakai and Zhang, 2021).

Another main message is that nutrient reductions have not always resulted in enhanced nutrient limitation in tidal waters of Chesapeake Bay (*e.g.*, TF2.3). This may be because (1) the phytoplankton are still mainly limited by light availability (or other factors), (2) external nutrient loads have not been reduced sufficiently to lower the estuarine nutrient concentrations, (3) internally accumulated nutrients are delaying the response of water column concentrations to reductions of external inputs (Jones, 2020), and/or (4) top-down controls of phytoplankton by grazers (Cohen et al., 1984) or competition for nutrients from submerged aquatic vegetation (Duarte, 1995; van Gerven et al., 2015) only occasionally limit phytoplankton growth at these tributary stations. We note that different locations of the tributaries can exhibit divergent trajectories and responses. Therefore, restoration may be most effective if strategies are tailored to locally specific characteristics (Wang et al., 2016; Zhang et al., 2022a). While management actions toward reducing nutrients from point and atmospheric sources seem to have resulted in the greatest water-quality improvements so far, continued nutrient reductions under the Bay TMDL, especially nonpoint sources, are critical to restoring the Bay and its tributaries (Ator et al., 2020; Hyer et al., 2021; Sabo et al., 2022; Zhang et al., 2022a, 2022b).

6. Conclusions

Large-scale nutrient reduction goals have been in place for decades as targets to restore water quality and habitat health in Chesapeake Bay and many other estuaries worldwide (Boesch, 2019; Cloern, 2001; Kemp et al., 2009; Malone and Newton, 2020; Smith, 2003). Management actions resulting in reductions in nutrient loads are expected to lead to enhanced nutrient limitation of phytoplankton growth, but it was unclear whether nutrient limitation has changed in the tidal tributaries following decades of reduction efforts. In this context, we demonstrate that machine learning approaches (*i.e.,* CART) can provide an innovative analysis tool to combine historical data from bioassay experiments with data from the tidal water-quality monitoring network for enhancing water-quality characterization and assessing water-quality response to management actions. Specifically, we have extended the previous work of Zhang et al. (2021) to develop CART models for analyzing nutrient limitation in three tributaries of Chesapeake Bay. CART satisfactorily reproduced the bioassay-based nutrient limitation patterns at the tributary stations (classification accuracy = 96%). Such an approach can greatly expand our ability to characterize nutrient limitation from the long-term tidal water-quality monitoring data, both for characterizing current conditions and for predicting temporal changes in nutrient limitation. Our results demonstrate long-term recovery trajectories in the tidal tributaries to Chesapeake Bay, which have management implications for Chesapeake Bay and other ecosystems. Overall, this research provides a new analytical tool for detecting changes in ecosystem functioning from long-term monitoring data and presents signs of ecosystem recovery following nutrient reductions. The approach can be adapted to other waterbodies, where bioassays and water-quality data sets are available, to detect ecosystem recovery (e.g., Tamminen and Andersen 2007).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data and code that support the findings of this study are available in Zhang (2022).

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.119099.

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Further reading

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